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ANNALS

OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

ANNALS OF HEAD & NECK MEDICINE & SURGERY

— Founded in 1892 —

34

THYROPLASTY: A NEW APPROACH

EXPERIMENTAL ENDOLYMPHATIC HYDROPS AND GLYCEROL

SITES OF PREDILECTION IN RECURRENT RESPIRATORY PAPILLOMATOSIS

CLASSIFICATION OF CONGENITAL MIDDLE EAR ANOMALIES

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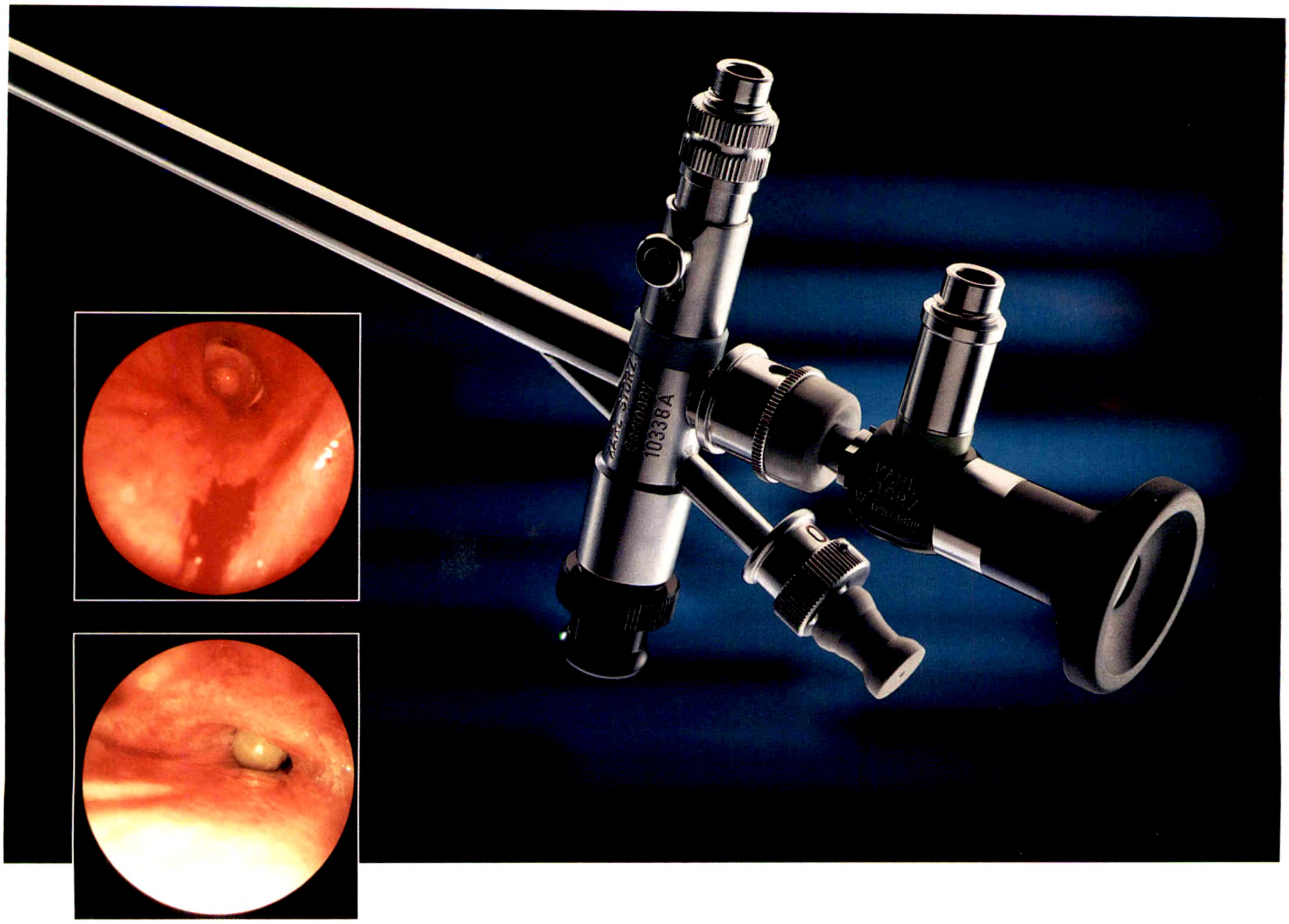

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THYROPLASTY: A NEW APPROACH

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NEW YORK, NEW YORK

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BOSTON, MASSACHUSETTS

A new type of thyroplasty implant (prosthesis) has been designed to be used as part of a surgical operation that accomplishes medial displacement of a paralyzed vocal cord. The implant is inserted through a rectangular window fashioned in the thyroid lamina on the side of the vocal cord paralysis. Instruments for measuring the location and outline of the window in the thyroid lamina are presented. A thyroplasty saw blade is introduced. By using this blade, a window of precise measurement can be constructed without injury to the underlying perichondrium. The implant is constructed from a biocompatible, medical-grade polymer. Four sizes each are available for the male and female larynges. The proper-size implant can be selected with a measuring device. It is not necessary to alter the implant at the time of surgery; it snaps into place and suturing is not necessary. Once the implant is inserted, its medial or lateral displacement is not possible; on the other hand, the implant can be easily removed if vocal cord function returns, or if a change to a different-size implant is indicated. The design of the implant will close the posterior commissure.

KEY WORDS — laryngeal framework surgery, phonosurgery, thyroplasty, vocal cord paralysis.

Two and a half decades ago, I (W.W.M.) was a member of the first group of laryngologists to use Teflon paste (polytef [PTFE], Mentor Division of Codman and Shurtleff, Inc, Randolph, Mass) as a method of medializing a paralyzed vocal cord.[1,2] After a few years of its usage, I concluded that 81% of my patients enjoyed improvement in vocal function; however, after 2 decades' experience with Teflon paste and further evaluation of my patients, I found that only 60% had good voices. Following a discussion with Isshiki and Blaugrund, 2 others experienced in this field,[3-9] I began using the thyroplasty type I operation as an alternate method for treating unilateral vocal cord paralysis in the paramedian and abducted positions.

Four years ago, I became disenchanted with the current techniques for the thyroplasty type I operation. There were 4 reasons for this. 1) Once the operation was performed, and vocal cord function subsequently returned, it was sometimes difficult to reverse the operation. 2) If, following the thyroplasty type I operation there was either undercorrection or overcorrection of the defect, adjustments were also difficult. 3) The cartilage trapdoor or silicone blocks that are used to medialize the paralyzed vocal cord are firm and nowhere near the consistency of the surrounding tissues. 4) Most of the thyroplasty type I procedures do not allow for closure of the posterior commissure.

With the above thoughts in mind, I began research using fresh human cadaver larynges (both male and female) along with skeletons of the laryngeal framework to find solutions to these problems. As a result of this research, a new type of thyroplasty prosthesis has been developed, to be used as part of a surgical operation that accomplishes medial displacement of the paralyzed vocal cord.

In order to facilitate proper localization and outlining of the window in the thyroid lamina, as well as to shorten the length of the operation, we are introducing 2 new instruments.

ADVANTAGES OF THYROPLASTY PROSTHESIS

1. The implant will be readily available, and thus it is not necessary to either fashion or alter it at the time of surgery. There are 4 sizes for the male larynx and 4 sizes for the female larynx.

2. By using a measuring device, the correct size can be easily determined intraoperatively by visualizing the larynx with a fiberoptic laryngoscope and listening to the patient's phonatory function.

3. The implant is easily inserted. It snaps into place and no sutures are necessary.

4. Once the implant is inserted, the chance for medial or lateral displacement is remote.

5. If indicated, the implant can be easily removed without damage to either the cartilaginous or soft tissue structures.

From the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, Massachusetts (Montgomery, Varvares), and the Department of Otolaryngology, Lenox Hill Hospital, New York, New York (Blaugrund).

Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 11-12, 1992.

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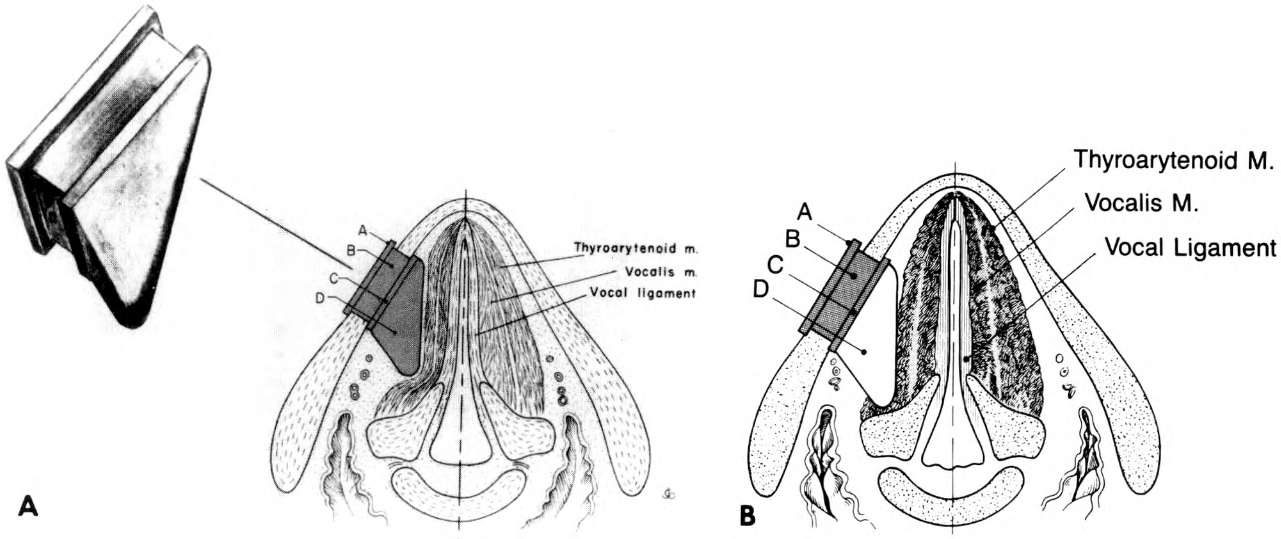


Fig 1. Design of thyroplasty implant. A) Thyroplasty implant consists of 2 components: tiered base and obtuse triangular top. There are 4 sizes for both male and female larynges. Difference in size relates to various heights of triangular portion of implant. Tiered base remains constant in all male and also female implants. B) Base of implant is made up of 3 tiers. Lateral portion of base (A) measures 9 x 14 x 1 mm for men and 7 x 12 x 1 mm for women and remains outside thyroid cartilage, on its surface. This design prevents inward displacement of implant. Middle portion of base (B) has same dimensions as window in thyroid lamina (7 x 12 x 3 mm for men and 5 x 10 x 2 mm for women). This portion of base stabilizes implant against any movement. Medial portion of base (C) remains inside thyroid lamina and is 7 x 16 x 1 mm for men and 5 x 14 x 1 for women. This design prevents outward displacement of implant. Base is constructed from firm, medical-grade material designed for long-term implantation (65 on durometer scale). Triangular intralaryngeal portion (D) of implant is designed to displace vocal cord medially in order to improve phonation. Height of this portion varies in size according to sex of patient and how far vocal cord is to be medially displaced. Posterior portion projects slightly posteriorly in order to reach base of vocal process of arytenoid. Medial margin of triangular intralaryngeal portion is rounded in order to reduce any potential trauma to vocal cord. Effective height of triangle varies depending on size of implant. Heights for women are 6 mm, 7 mm, 8 mm, and 9 mm. Heights for men are 8 mm, 9 mm, 10 mm, and 11 mm. Lateral aspect (base) of triangular intralaryngeal portion adheres to medial tier of base. For women, this dimension is 5 x 12 mm, and for men, 7 x 14 mm. Obtuse-triangular, intralaryngeal portion is constructed with softer (30 on durometer), radiopaque, medical-grade material suitable for long-term implantation.

6. Because of the uniformity of the base design, different-size implants can be inserted with no change in the thyroid lamina window.

7. The design of the implant will close the posterior commissure, giving a higher percentage of good postoperative results.

DESIGN OF THYROPLASTY PROSTHESIS

Basically, there are 2 parts to the prosthesis (Fig 1). The lateral, or outer, portion serves to lock the prosthesis to the window made in the thyroid lamina. This lateral portion is constructed with 3 rectangular plates of firm material. The most lateral plate (A) remains on the outer surface of the thyroid lamina and prevents medial displacement of the prosthesis. The middle portion (B), which is the same size as the window in the thyroid lamina, stabilizes the implant against any movement. The inner plate (C) remains against the inner surface of the thyroid lamina and serves to prevent lateral displacement of the prosthesis. The lateral portion remains constant for both the male and the female larynges. The lateral portion of the prosthesis must be quite firm, but on the other hand, malleable enough to allow for flexibility during its insertion. A density of 65 on the durometer scale (Shorea) was found to be best suited for this purpose.

The inner, or medial, portion (D) of the prosthesis, which serves to medialize the vocal cord and the vocal process of the arytenoid, is of a rather complex design. It is triangular in shape. The hypotenuse of this triangle is designed to correspond to the long axis of the vocal cord when it is in the median position. The base of the triangle is adherent to the inner plate of the portion of the prosthesis that is fixed to the thyroid lamina. The third side of the triangle makes a 116° angle to the plane of the base. Its tip thus projects posteriorly in order to contact the base of the vocal process of the arytenoid. In so doing, it accomplishes closure of the posterior commissure.

It was necessary to determine the type of polymer and the density thereof for the medial, triangular portion of the prosthesis. This research involved working with a medical products manufacturer (Boston Medical Products, Waltham, Mass), including a polymer chemist, in its research and development laboratory. We concluded that a biocompatible, medi-

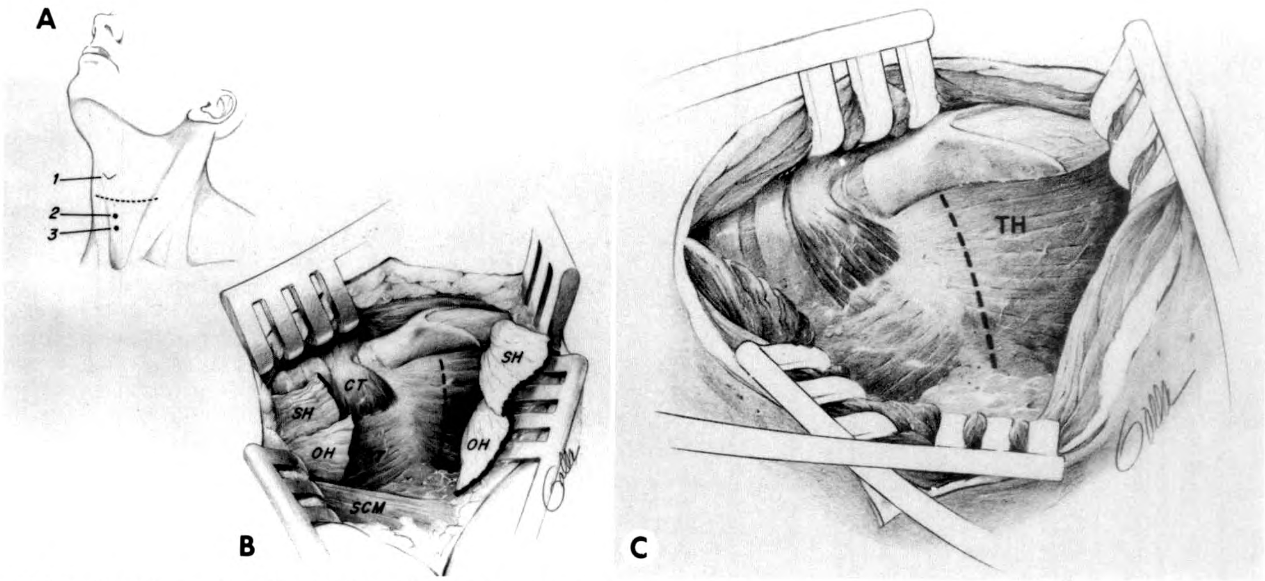


Fig 2. A) Oblique view of neck with horizontal skin incision. Thyroid notch (1), cricothyroid membrane (2), and inferior margin of cricoid cartilage (3) are identified in anterior midline of neck. Incision begins on contralateral side of neck, midway between thyroid notch and inferior margin of thyroid cartilage. It is extended on ipsilateral side to just beyond anterior border of sternocleidomastoid muscle. B) Skin flaps are established superiorly and inferiorly in plane above fascia covering strap muscles. Two sternohyoid muscles are separated in midline, exposing thyroid notch, thyroid lamina anteriorly, and cricothyroid membrane. As general rule, sternohyoid and omohyoid (anterior belly) muscles are retracted laterally, exposing thyrohyoid muscle. SH — sternohyoid, CT — cricothyroid, OH — omohyoid, ST — sternothyroid, SCM — sternocleidomastoid. C) Broken line indicates level for transecting thyrohyoid muscle (TH). This is incised, exposing thyroid lamina and inferior thyroid tubercle. On occasion, exposure can be difficult. In such cases, strap muscles can be transected. This adds little or nothing to postoperative morbidity of operation.

cal-grade polymer would be the ideal material, one that could be readily fused to the outer portion of the prosthesis during processing. We determined that a density of 30 on the durometer scale (Shorea) would be most compatible with the surrounding tissues. This portion of the prosthesis is radiopaque. The overall contour of the triangle portion is designed so that it will medialize only the true vocal cord, and not the ventricle or false vocal cord. There are 4 sizes for the male larynx, and 4 sizes for the female larynx.

EVALUATION OF PATIENT

Preoperatively, the larynx is evaluated by videolaryngoscopy and videolaryngostroboscopy using the Olympus P3 fiberoptic scope and the Nagashima rigid scope. The voice is evaluated using the Visipitch. The parameters of this evaluation include amplitude, range, fundamental frequency, jitter, and maximum phonation time (MPT). This evaluation is repeated 4 to 6 weeks after the operation, and again 6 months and 1 year postoperatively.

ANESTHESIA

A competent anesthetist or anesthesiologist is essential for the success of this operation. The correct balance between sedation and responsiveness has to be maintained during the entire operation. The suggested anesthesia for thyroplasty in the average 70-kg patient is as follows. Initially, Versed, 1 to 2 mg intravenously, is used in the holding area. Fentanyl citrate, 50 µg/mL, is used in the holding area or on the way to the operating room. In the operating room, fentanyl, 25 µg (x2), is used at the beginning of the operation. Incremental doses of propofol are used 30-20-10 mL, etc.

One nasal cavity is sprayed with a mixture of 3% Xylocaine and 1% phenylephrine hydrochloride and then packed with a pledget saturated with 4% cocaine hydrochloride solution. The pack remains in place until the introduction of the fiberoptic laryngoscope. The line of the incision and the underlying musculature are injected with 2% Xylocaine and 1:100,000 ephedrine hydrochloride.

Two to 5 minutes prior to insertion of the prosthesis, 50 to 100 mg of lidocaine hydrochloride is used, followed by propofol, after laryngeal evaluation with a fiberoptic scope inserted through the nose.

TECHNIQUE OF SURGERY

The horizontal incision begins on the contralateral side of the neck, midway between the thyroid notch and the inferior margin of the thyroid cartilage. It extends to the ipsilateral side of the neck, just beyond the anterior border of the sternocleidomastoid muscle (Fig 2A). Skin flaps are established superiorly and

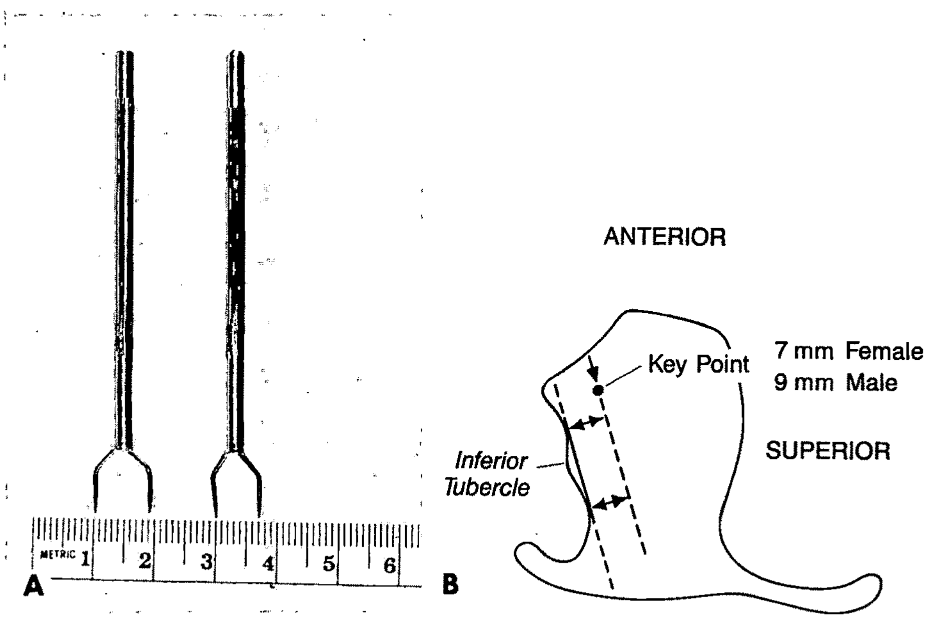


Fig 3. Orientation of window. A) Measuring device 1. Instruments used to measure from inferior border of thyroid cartilage to superior border of thyroplasty window, and from anterior midline of thyroid cartilage to anterosuperior angle (key point) of thyroplasty window. B) Inferior thyroid tubercle is palpated about midway along inferior border. Marks are made with calipers (measuring device) directly superior from points anterior and posterior to inferior thyroid tubercle. Line connecting these 2 points indicates direction of vocal cord and is about midway between thyroid notch and cricothyroid membrane. Line marks superior border of window. Anterosuperior point (key point) is located by measuring with calipers or measuring device from anterior midline and along superior border of window.

inferiorly in a plane superficial to the fascia covering the strap muscles. The sternohyoid muscles are separated in the midline so as to identify the thyroid notch, the thyroid lamina anteriorly, and the cricothyroid membrane.

The strap muscles are undermined and retracted laterally (Fig 2B); however, if exposure is difficult, they are transected at a level midway between the thyroid notch and the cricothyroid membrane (Fig 2B). Exposure is accomplished with 2 Wheatlander-type self-retaining retractors. Most often, it is not necessary to transect the strap muscles.

The thyrohyoid muscle is transected (Fig 2C) so as to expose the thyroid lamina. The inferior thyroid tubercle is palpated about midway along the inferior border of the thyroid lamina. It is exposed with sharp and blunt dissection (Fig 2C).

Instruments have been designed to accurately locate and outline the window in the thyroid lamina (Fig 3A). These measuring devices (7 mm in women and 9 mm in men) are used to locate the superior border and the anterosuperior angle (key point) of the window (Fig 3B). After the inferior thyroid tubercle has been defined and exposed, the inferior border of the thyroid lamina is exposed anterior and posterior to this structure. One point of the calipers touches the inferior border of the thyroid lamina, anterior to the inferior thyroid tubercle, and the other, a point directly superior. As the inferior point is lifted free, a Bovie is applied to the tip of the measuring device and a cautery mark will appear at the superior point. A similar cautery point is made beginning on the inferior border, posterior to the inferior thyroid tubercle. The anterior and posterior cautery marks are connected with a marking pen. This line is extended to the anterior aspect of the thyroid lamina. With the same calipers, a mark is made along the line, beginning at the anterior midline and extending posteriorly. This cautery mark is the point of the anterosuperior angle (key point) of the thyroid lamina window. The distance again is 7 mm in the female and 9 mm in the male larynx.

OUTLINE OF WINDOW

The instrument for making the outline of the window is basically a modified cautery tip ("branding iron"; Fig 4A). The instrument is first inserted into the Bovie handle, with the tip placed so that the anterosuperior angle and superior border correspond to the "key point" mark and line on the thyroid lamina. Current is applied and 4 marks appear on the cartilage surface (Fig 4B). These marks are connected with a marking pen, thus completing the outline of the window.

Conventional Method for Outlining Window. The measurements for constructing the window in the thyroid lamina for the purpose of thyroplasty type I are summarized in Fig 5. The anterosuperior angle of the window is measured from the anterior midline along the anteroposterior line with calipers. This measurement is 7 mm for the female larynx and 9 mm

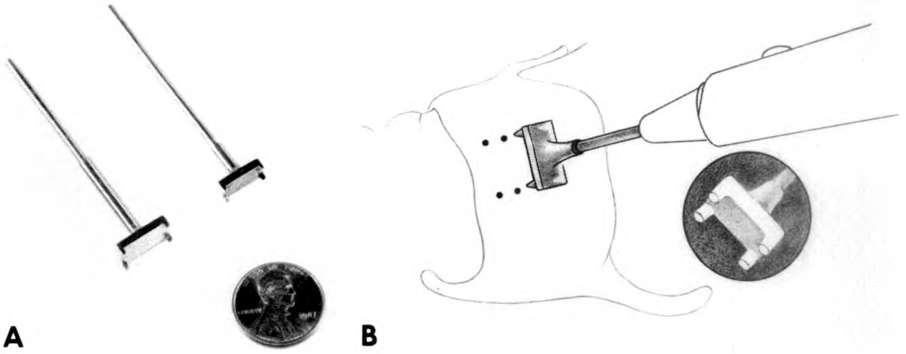


Fig 4. Measuring device 2. A) Modified cautery tip used to outline window. B) Once line of superior border and anterosuperior point (key point) of window has been established on thyroid lamina, modified cautery tip that measures 5 x 10 mm in women and 7 x 12 in men can be used as shortcut. Four cautery marks are connected, completing outline of window.

for the male. The anteroinferior angle of the window is measured directly inferior from the first point. This measures 5 mm for the female larynx and 7 mm for the male. The posterosuperior angle of the window is measured posteriorly along the line from the anterosuperior point. This measures 10 mm for the female larynx and 12 mm for the male. The posteroinferior angle of the window is made directly inferior from the posterosuperior point. This measures 5 mm for the female larynx and 7 mm for the male. It is again emphasized that the superior and inferior borders of the window must be parallel to the inferior border of the thyroid lamina. The 4 points are connected to complete the rectangular window outline.

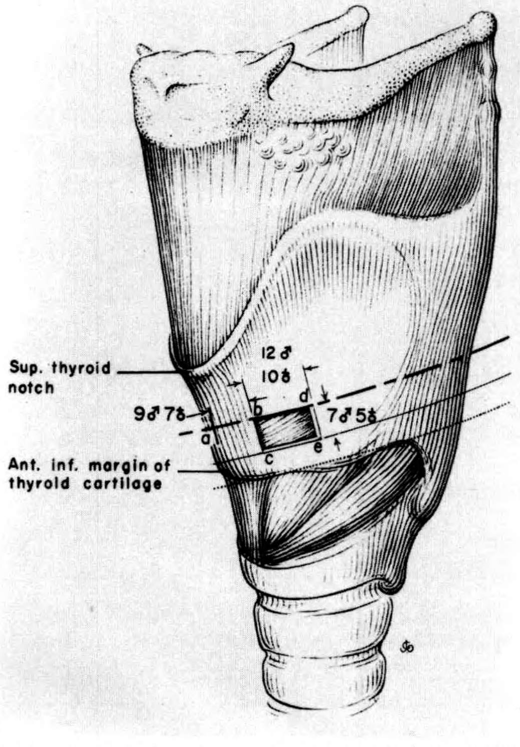


Fig 5. Design of window (conventional method). With calipers, anterosuperior angle (same as key point, b) for thyroid lamina window is measured from anterior midline (a). This measurement (a-b) is 7 mm for female and 9 mm for male larynx. Anteroinferior angle (c) for window is measured from point b. This measurement (b-c) is 5 mm for female and 7 mm for male larynx. Posterosuperior angle (d) for window is measured from anterosuperior point (b). This measurement (b-d) is 10 mm in female and 12 mm in male. Posteroinferior angle (e) is made from posterosuperior point (d). This measurement (d-e) is 5 mm in female and 7 mm in male larynx. It is again emphasized that superior and inferior borders of window must be parallel to inferior border of thyroid lamina. These 4 points are connected to complete window outline.

Method for Cutting Window. A small, tangential electric saw (Fig 6A) has been specially designed for the thyroplasty type I operation and other laryngeal framework surgery (Stryker Corp). The outer perichondrium is not disturbed. This saw can be used in all cases, regardless of whether or not the cartilage has undergone ossification. Cutting the window (Fig 6C) is accomplished with great care so as not to injure the underlying soft tissues. Once the window is free, it is carefully separated from the underlying perichondrium with a curved periosteal elevator and removed.

INNER PERICHONDRIUM

Following removal of the cartilage from the window, the inner perichondrium is elevated from the underlying cartilage in all directions (Fig 7A). A midline anteroposterior line is cauterized with bipolar cautery on the inner perichondrium as indicated in Fig 7B. This line in the inner perichondrium is carefully incised so as to expose the underlying fascia covering the intrinsic laryngeal musculature. Sharp Stevens scissors work well for making this incision. It is my impression that medialization is not as precise when the inner perichondrium is not incised. If the inner perichondrium remains intact, it is tented in by the prosthesis, and unnecessary and undesired medialization is accomplished superiorly and inferiorly.

MEASURING FOR IMPLANT

A measuring device (Fig 8) is inserted through the window in the thyroid lamina. There are 4 sizes for

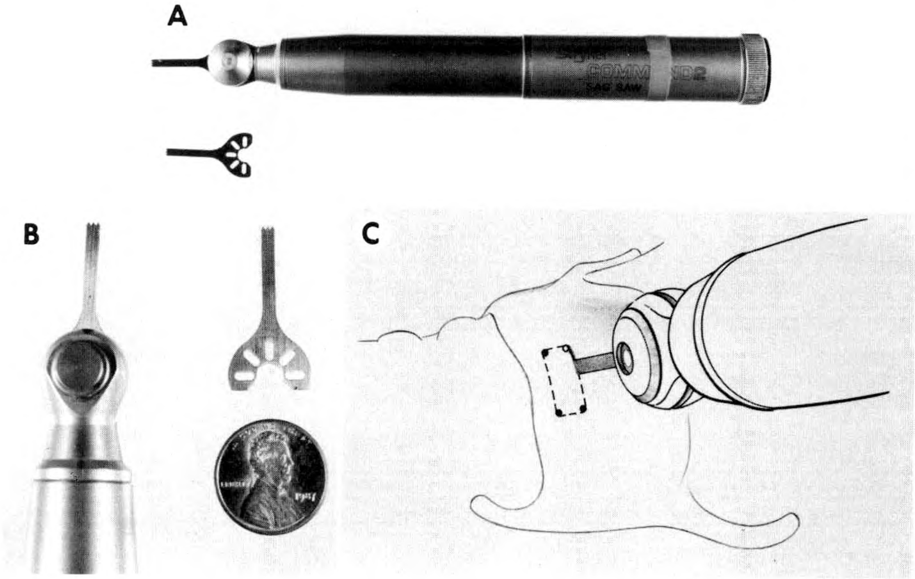


Fig 6. Thyroplasty saw. A) Photograph of thyroplasty saw handle and blade (Stryker Corporation, Kalamazoo, Mich). B) Small electric thyroplasty saw handle and blade have been specially designed for laryngeal framework surgery. C) Cutting this window is accomplished with care so as not to injure underlying soft tissues. Cartilage is carefully separated from underlying perichondrium with curved periosteal elevator and is removed.

both the male and female larynges. The vocal cords are observed with a fiberoptic laryngoscope during this process. The end point is complete closure of the vocal chink, from the anterior commissure to the posterior commissure. The ideal measurement is recorded so that the correct-size implant can be selected.

INSERTION OF IMPLANT

The thyroplasty implant is grasped with forceps (Fig 9A). The posterior tip of the medial triangular portion of the implant is inserted through the window. The slot in the tiered base of the implant is engaged into the posterior rim of the window in the thyroid lamina. A duck-bill spatula is inserted into the slot anteriorly, and the prosthesis is snapped into place (Fig 9B).

If the implant requires change at the time of surgery, or change or removal at a later date as a secondary procedure, the anterior end of the external portion of the implant (lateral tier) is grasped with forceps or a hemostat. With lateral pull, the implant snaps out as easily as it was inserted.

REPAIR

The muscle (if transected) and subcutaneous tissues are approximated with 3-0 chromic catgut, and the skin incision is repaired with 6-0 mild chromic catgut. Steristrips are applied after preparing the skin with Mastosol liquid adhesive or tincture of benzoin. Butterfly-closed suction drainage is used along with red-top vacuum tubes.

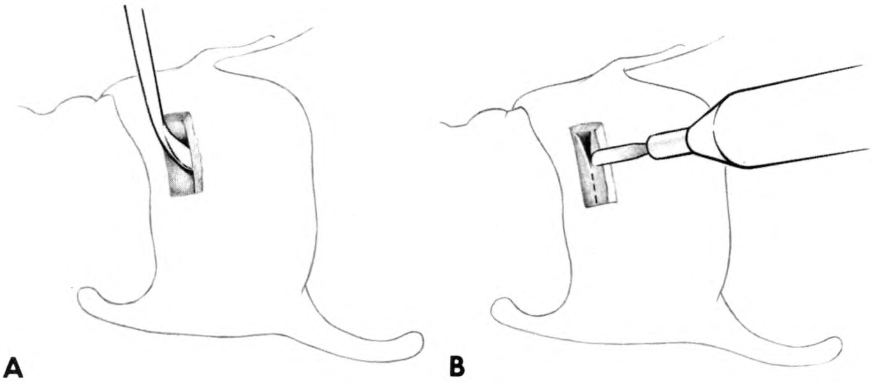
POSTOPERATIVE CARE

1. Medication for pain may be needed the first day.

2. Antibiotics, which were started preoperatively, are continued for 1 week. Intravenous Decadron (8 to 12 mg) is given at the beginning of the operation and continued for 2 additional doses at 8-hour intervals.

3. Red-top vacuum tubes are changed at 2- to 4-hour intervals or as soon as they are one-fourth filled with blood. This requires careful nursing surveil-

Fig 7. Inner perichondrium. A) Once cartilage window has been removed, inner perichondrium is elevated from cartilage in all directions. B) Midline anteroposterior line is cauterized in inner perichondrium with Bovie cautery on low current or bipolar cautery. This line is incised carefully with delicate scissors (Stevens) so that underlying fascia and muscle tissue are not injured.



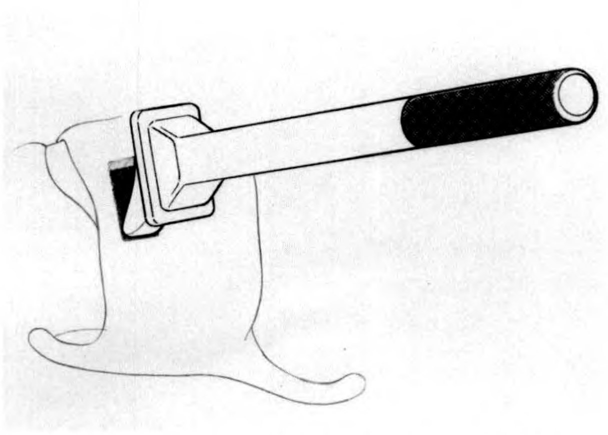


Fig 8. Measuring for implant. Implant sizes are 6, 7, 8, and 9 mm for women and 8, 9, 10, and 11 mm for men. Measuring device is shown being inserted through window in thyroid lamina. There are also 4 measuring devices for both male and female larynges. Measuring device is inserted while vocal cords are observed with fiberoptic laryngoscope. Ideal measurement is recorded so that correct-size implant can be selected.

lance.

4. The butterfly-closed suction drainage tubing is removed the morning following surgery, and the patient is discharged from the hospital. Same-day discharge is not advised because of the possibility of laryngeal edema or intralaryngeal bleeding that could interfere with the laryngeal airway.

5. The medial portion of the implant is radiopaque; thus, if indicated, the exact position of the prosthesis can be easily determined with a computed tomographic scan at the level of the vocal cords by a few 2-mm cuts, along with coronal reconstruction.

6. It is my opinion that evaluation of the patient by videofibrolaryngoscopy or videolaryngostroboscopy and voice analysis is of little value during the immediate postoperative period. The patient is thus evaluated in 4 to 6 weeks and again at 6 months and at 1 year.

RESULTS

The last 54 patients have undergone thyroplasty with the final, perfected model of this thyroplasty implant. There were 31 men and 23 women. The age range was from 21 to 85 years, with a mean age of 51.2 years. All patients had unilateral vocal cord paralysis, 27 on the right side and 27 on the left. The causes of paralysis were as follows: intracranial pathology 6, idiopathic 12, previous neck surgery 17, intrathoracic surgery 7, skull base lesion 8, neck trauma 2, intrathoracic neoplasia 1, and hemilaryngectomy 1.

A total of 56 procedures were performed in the 54

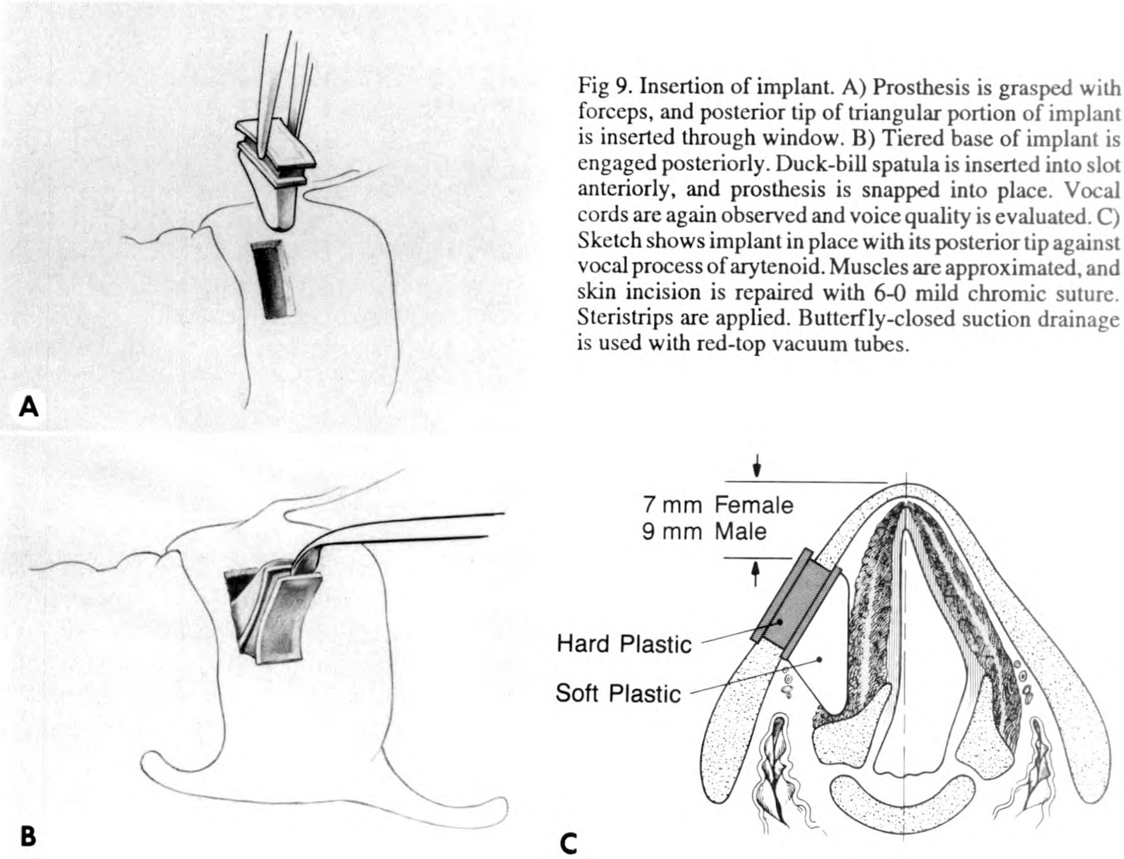


Fig 9. Insertion of implant. A) Prosthesis is grasped with forceps, and posterior tip of triangular portion of implant is inserted through window. B) Tiered base of implant is engaged posteriorly. Duck-bill spatula is inserted into slot anteriorly, and prosthesis is snapped into place. Vocal cords are again observed and voice quality is evaluated. C) Sketch shows implant in place with its posterior tip against vocal process of arytenoid. Muscles are approximated, and skin incision is repaired with 6-0 mild chromic suture. Steristrips are applied. Butterfly-closed suction drainage is used with red-top vacuum tubes.

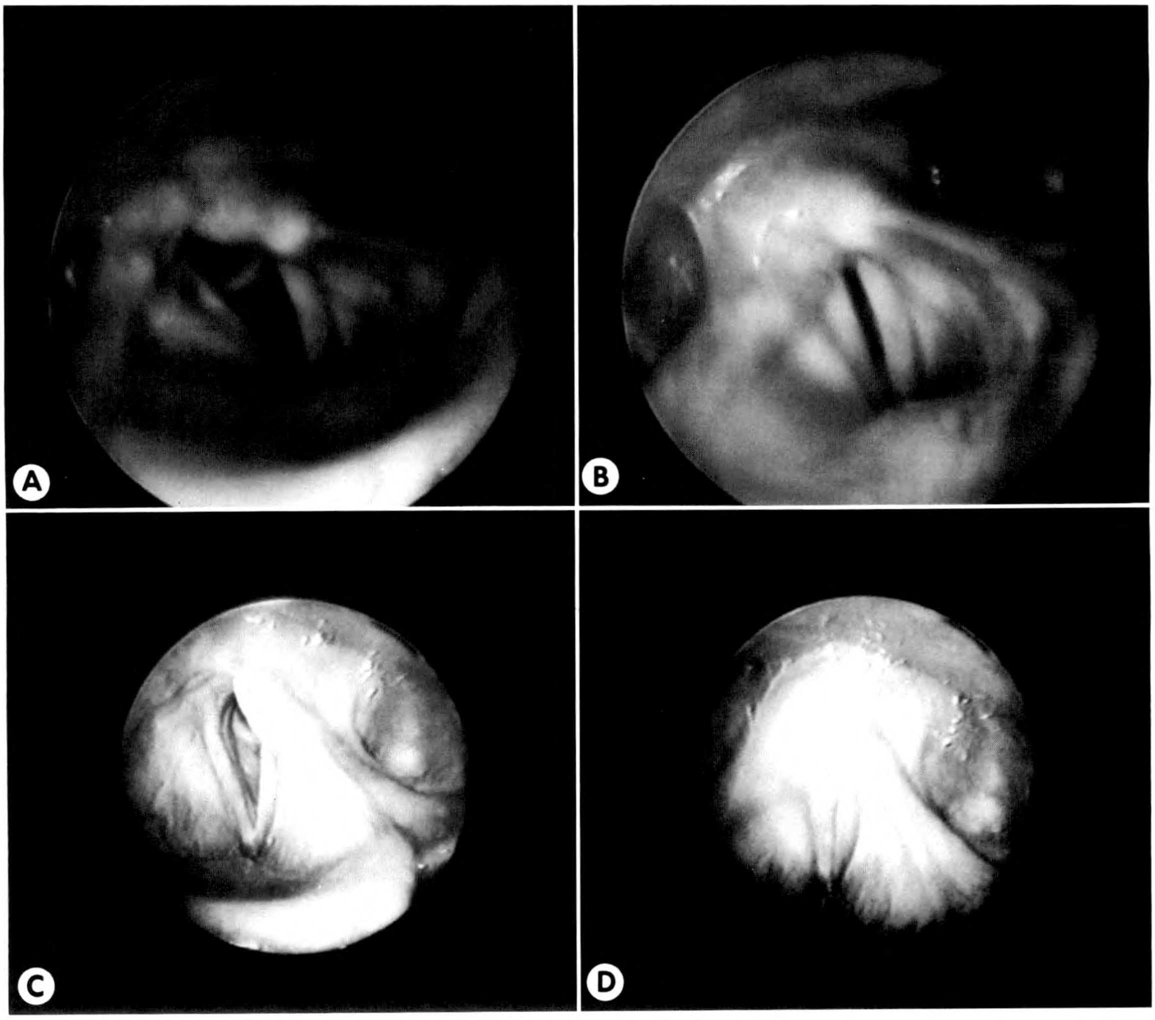


Fig 10. Left vocal cord paralysis. A,B) Preoperative photographs taken during A) inspiration and B) phonation. C,D) Six months postoperatively, during C) inspiration and D) phonation.

patients, with 2 patients each requiring 1 revision. Fifty-three patients had a subjective improvement in their voice as determined by the patient's and surgeon's assessment of voice postoperatively and the surgeon's assessment of glottic closure on laryngeal examination.

There were 2 complications perioperatively. Two patients developed laryngeal edema requiring treatment with steroids and antibiotics. There were no other complications in this group.

The 2 patients who underwent revision did so to allow placement of a larger prosthesis. At surgery, the tissue adjacent to the previously implanted prosthesis showed a thin fibrous capsule formation and no gross evidence of inflammation.

Three patients in this group had implantation performed following radiotherapy to the head and neck. There has been no difficulty noted in those patients related to placement of the implant into an irradiated field. One patient had the implant placed during radiotherapy to the neck, with no ill effects.

Figure 10 illustrates a representative case. This patient suffered a left vocal cord paralysis during neck surgery for melanoma. He has undergone a thyroplasty using this technique. Figure 10A shows the vocal cords preoperatively in the position of abduction; Fig 10B, in the position of adduction; Fig 10C, postoperatively in the position of abduction; and Fig 10D, postoperatively in the position of adduction. Note that the posterior commissure is closed after placement of the thyroplasty implant.

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The 1994 Midwinter Meeting of the Association for Research in Otolaryngology will be held in St Petersburg Beach, Florida, February 6-10, 1994. For information, contact Michele Johnson, Executive Secretary, 431 E Locust St, Suite 202, Des Moines, IA 50309; telephone (515) 243-1558; fax (515) 243-2049.

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SITES OF PREDILECTION IN RECURRENT RESPIRATORY PAPILLOMATOSIS

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Florid and widespread respiratory papillomatosis is a devastating disorder occurring in a subset of patients with recurrent respiratory papillomatosis, and it poses a major dilemma for the patient and the surgeon. Contrary to common belief, the distribution of papilloma lesions is not random, but follows a predictable pattern, with lesions occurring at anatomic sites in which ciliated and squamous epithelia are juxtaposed. The predominant sites of disease in recurrent respiratory papillomatosis are the limen vestibuli, the nasopharyngeal surface of the soft palate, the midzone of the laryngeal surface of the epiglottis, the upper and lower margins of the ventricle, the undersurface of the vocal folds, the carina, and bronchial spurs. These sites have the common histologic feature of a squamociliary junction. Papillomata also occur at the tracheostomy tract and at the midthoracic trachea in patients with tracheostomies. At the latter sites, abrasion injury to ciliated epithelium heals with metaplastic squamous epithelium and creates an iatrogenic squamociliary junction. The apparent preferential localization of papilloma at squamociliary junctions has at least 2 implications: first, that detection of occult asymptomatic papillomata is enhanced by careful examination of squamociliary junctions, and, second, that iatrogenic papilloma "implantation" is preventable by avoiding injury to nondiseased squamous and ciliated epithelia.

KEY WORDS — epithelium, human papillomavirus, recurrent respiratory papillomatosis, sites of predilection.

INTRODUCTION

Respiratory papilloma, a histologically benign neoplasm, can be either solitary or, in its most troublesome form, multiple; the lesions are caused by human papillomavirus (HPV) types 6 and 11. The growth and spontaneous regression of papillomas are unpredictable, but recurrent growth after apparent complete endoscopic excision has frustrated generations of otolaryngologists. The common view is that recurrent respiratory papilloma (RRP) lesions are randomly distributed throughout the respiratory tract without apparent pattern. This report has been stimulated by the clinical observation that papilloma distribution is not random, but rather, the lesions occur at sites with a squamous-ciliary junction.

Molecular hybridization techniques have identified over 60 HPV types.[1] Types 6[2] and 11[3] are associated with respiratory tract papillomatosis, and specific viral subtypes may be correlated with disease severity and clinical course.[4] The present understanding is that HPV establishes itself in the basal layer, where viral DNA enters the cell, and elaborates RNA to produce viral proteins.[5] Early viral gene expression in the stratum spinosum is associated with acanthosis and cellular proliferation. In the mature layers of epithelium, late genes direct production of proteins related to capsid assembly. Identification of HPV virions by the peroxidase-antiperoxidase technique has demonstrated internal capsid antigen in the mature outer layers of the epithelium,[6-8] and in situ hybridization demonstrates HPV DNA and RNA in the basal and parabasal cells.[8]

Human papillomavirus DNA has been demonstrated in clinically normal epithelium of patients with active RRP and also in patients during clinical remission.[9] This finding identifies a potential virus reservoir for reinfection and new lesion production.

CLINICAL SITES OF DISEASE

A composite of published reports of disease sites in over 400 RRP patients indicates that the larynx is diseased in the largest proportion of cases; the true vocal folds, epiglottis, and false vocal folds are the sites most often specified (see Table[10-18]). In a 66-patient multicenter RRP study,[19] these same laryngeal sites were named. In the lower respiratory tract the distribution pattern occurs in 2 forms. Among those patients with a tracheostomy, papillomata were present at the mucociliary junction at the tracheostomy site, and in the midthoracic trachea, at a level corresponding to the tip of the tracheostomy tube. Pulmonary papillomatosis is observed in the peripheral lung field. In patients without a tracheostomy, papilloma distal to the immediate undersurface of the vocal folds is unusual.

A pilot survey to discover occult sites of asymp-

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SITES OF PAPILLOMA PREDILECTION

	No. of Patients With Site Involved	*Sample Size*	*Frequency of Involvement (%)*	*References*
Nasal vestibule	7	269	2.6	10,11
Naso-pharynx	8	269	3.0	10,11
Palate	37	359	10.3	10-12
Epiglottis	6	52	11.6	13
Larynx	417	426	97.9	10-14
Trachea	37	339	10.9	10,12-16
Bronchi	20	385	5.2	12,14-17
Lung	13	515	2.5	10-12,15,17
Tracheos-tomy site	25	65*	38.4	18

*Number of patients with tracheostomy.

tomatic papilloma noted an unexpectedly large number of lesions in the nasopharynx and the undersurface of the vocal folds. Papilloma at these sites was present in approximately 15% of RRP patients.[19] The nasal vestibule is an additional site of papilloma occurrence, usually as an isolated lesion, and infrequently as part of widespread RRP.

Each of the foregoing sites exhibits the common histologic feature of a squamociliary junction.

HISTOLOGY AT SITES OF PREDILECTION

Ciliated, so-called respiratory, epithelium covers virtually all of the upper airway from the nasal vestibule to the peripheral lung. Cells in this specialized epithelium have 50 to 200 cilia per cell; synchronized ciliary beating transports mucous secretions, which consist of a stationary liquid phase overlain by a mobile gel phase, at sites exposed to trauma of abrasion, impact, or pressure. Traumatized ciliated epithelium is replaced by squamous epithelium, and a squamociliary junction is created.

ANATOMIC SITES OF PAPILLOMA PREDILECTION

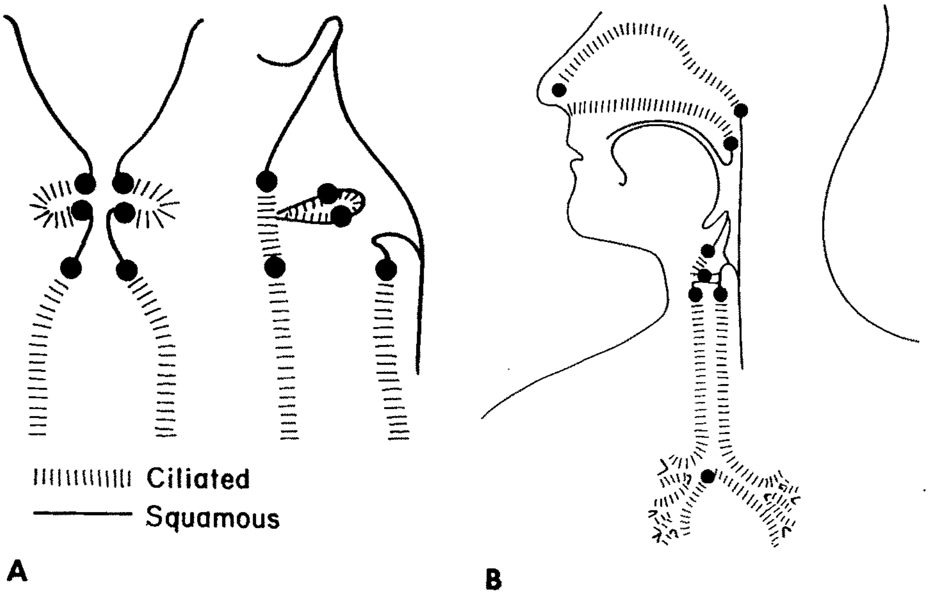
Specific anatomic sites in which RRP lesions occur will be enumerated (see Figure).

Anterior Nares. The squamous epithelium of the skin continues into the anterior nares, where it forms a junction with the ciliated epithelium lining the nasal cavity. The nasal mucociliary stream is directed posteriorly, except at the anteriormost 1 to 2 cm, where mucous flow is toward the nares and mucous secretions are transported to the limen vestibuli.

Nasopharynx. The ciliated epithelium of the nasal cavity continues onto the superior (nasopharyngeal) surface of the soft palate, where it meets the squamous epithelium lining the lower portion of the palatal nasopharynx, which coapts with the posterior wall of the nasopharynx during velopharyngeal closure accompanying speech, swallowing, and cough.[20] Epithelium in the palatal nasopharyngeus is subject to abrasive trauma against the posterior oropharyngeal wall, and a squamociliary junction results.

Larynx. The upper half of the laryngeal surface of the epiglottis is lined by squamous epithelium, and the lower half by ciliated epithelium. The nonciliated upper half brushes the posterior pharynx during each swallow and is exposed to explosive airflow turbulence during cough. The lingual surface of the epiglottis is exposed to bolus flow and is lined by squamous epithelium. A squamociliary line sepa-

Squamous lining (solid line), ciliated lining (bars), and squamociliary junctions (round dots). See text for full description. A) Laryngeal epithelium. B) Respiratory tract epithelium.



rates the upper and lower halves of the epiglottis.

Vestibular Fold. The vestibular (false vocal) fold is lined by squamous epithelium on the upper surface and by ciliated epithelium on its undersurface (roof of ventricle). The vocal folds are lined by squamous epithelium, thickest along the free border and thinner on the upper surface of the vocal folds, where it joins the ciliated epithelium of the floor of the ventricle, and on the undersurface of the vocal folds, where it meets the ciliated tracheal epithelium.

Trachea and Bronchus. The tracheal and bronchial epithelia are ciliated. The proportion of ciliated cells diminishes from over 50% in the trachea to 15% in the fifth-generation bronchi.[21] The epithelia at the carina and at bronchial spurs have variable cell composition, and at some sites may be totally nonciliated, particularly in older subjects.

Lungs. Gradual diminution of ciliated epithelial cells is observed from the main stem bronchus through its successive divisions into the peripheral bronchioles. In the terminal bronchioles the epithelium is primarily of a cuboidal and columnar, nonciliated type. The alveolar sac is lined by flat, type 1 pneumocytes and cuboidal, type 2 pneumocytes. Ciliated, columnar, and squamous epithelia are not present in the alveoli.

DISCUSSION

Ciliated epithelium undergoes squamous metaplasia when exposed to the repeated trauma of impact, abrasion, irritation, or exposure. Such metaplastic epithelium, when located between the squamous and ciliated epithelia, has been termed transitional epithelium.[20] Injury to ciliated epithelium is usually repaired by nonciliated epithelium that creates an iatrogenic squamociliary junction. Thus, after tracheostomy, ciliated tracheal epithelium is juxtaposed to newly formed squamous epithelium lining the tracheostomy tract. Additional zones of squamous metaplasia and squamociliary junctions develop in the lower trachea, where the tracheotomy tube tip produces abrasive injury during each respiratory excursion. Injury to tracheal epithelium due to surgical instrumentation (suction tip, forceps, or laser impact) or prolonged endotracheal intubation also heals by squamous metaplasia and results in a squamociliary junction — a patch of squamous epithelium surrounded by normal ciliated epithelium.

The squamociliary junction is identified as the common histologic feature at anatomic sites of papilloma predilection. Sites exhibiting this histology appear to be at increased risk for papilloma formation; this hypothesis is supported by the occurrence of asymptomatic and occult papillomata at natural and iatrogenic squamociliary junctions, identified in this report. Inasmuch as HPV is the essential etiologic agent in papilloma formation, what factors favor establishment of active and productive infection at such sites?

An eddying flow of the mucous blanket at squamociliary junctions may concentrate the infectious virus particles at these sites. To our knowledge, HPV has not been demonstrated in ciliated epithelium, either because of a requirement of mature squamous epithelium for virus expression, or because of an absence of viral receptors in ciliated epithelium. Other, unspecified elements such as protective factors in mucous secretions, local immunity, and cell surface receptors may also be relevant to the apparent resistance of ciliated epithelium to HPV infection, but to date, these matters remain speculative.

On the basis of available evidence, the greatest number of virus particles is present in the papilloma lesions, and lesser amounts are detectable in adjacent, clinically normal epithelium.[9] Thus, surgical excision of papillomatous lesions reduces the reservoir of infecting virus, but does not achieve complete virus eradication.

The sites of epithelial injury, such as those that occur after surgical excision of papilloma, are at increased risk for reinfection by virus, and lesion "recurrence" occurs precisely at the same point. This propensity for papilloma to recur in an excision site or at a point of epithelial injury is well recognized, but poorly understood. It is probable that HPV DNA present in adjacent clinically and histologically normal epithelium migrates into the injured site and has a role in the formation of the "recurrence."

Squamous metaplasia in tracheal epithelium after tracheostomy creates an iatrogenic squamociliary junction in which papilloma development is regularly observed. Metaplastic squamous epithelium at this site appears highly susceptible to HPV infection, and papilloma is present at the tracheocutaneous junction in a high proportion of tracheotomized subjects. The occurrence of tracheal papilloma is invariably preceded by tracheostomy, and bronchial papilloma is preceded by tracheal papilloma, with rare exceptions. In the midthoracic trachea, papilloma deposits occur in 2 forms: sessile growths that occur on the posterior wall corresponding to the convex surface of the tracheotomy tube, and friable exophytic papillomata, which develop on the anterior wall, where the tip of the tracheotomy tube creates a troublesome obstructive lesion. Pulmonary papilloma is preceded by tracheobronchial papilloma.[15]

Complete clinical remission from papillomatosis probably does not require total eradication of viral DNA. Although viral DNA persistence in clinically normal tissue retains potential for disease reactivation, this is reminiscent of the herpes simplex virus infection, which remains clinically silent in host tissues for prolonged periods and can be reactivated following physical or psychological stress.[22] Human papillomavirus DNA remains clinically silent during complete remission, but retains potential for relapse such as in the immunocompromised or debilitated host, and probably after epithelial trauma.

The apparent preferential localization of papilloma at squamociliary junctions has at least 2 implications. First, the detection of occult asymptomatic papilloma can be enhanced by careful examination of sites with a squamociliary junction. Second, iatrogenic papilloma "implantation" is reducible by avoiding injury to nondiseased squamous and ciliated epithelium. Tracheobronchial papillomatosis rarely occurs without a prior history of tracheostomy. On the basis of this rationale, clinical management translates as follows. First, tracheostomy should be avoided whenever possible. Second, when tracheostomy is present, every effort should be made to achieve decannulation; we have observed reduced regrowth and regression of established lesions in such cases. Other factors that may inhibit papilloma development, such as host immune factors, natural and induced defense mechanisms, and antiviral and growth-inhibiting agents, have potentially important but presently undefined roles in the clinical course of this condition.

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MEASUREMENT OF YOUNG'S MODULUS IN THE IN VIVO HUMAN VOCAL FOLDS

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Currently, surgeons have no objective means to evaluate and optimize results of phonosurgery intraoperatively. Instead, they usually judge the vocal folds subjectively by visual inspection or by listening to the voice. This paper describes a new device that measures Young's (elastic) modulus values for the human vocal fold intraoperatively. Physiologically, the modulus of the vocal fold may be important in determining the nature of vocal fold vibration in normal and pathologic states. This study also reports the effect of recurrent laryngeal nerve stimulation on Young's modulus of the human vocal folds, measured by means of transcutaneous nerve stimulation techniques. Young's modulus increased with increases in current stimulation to the recurrent laryngeal nerve. Ultimately, Young's modulus values may assist surgeons in optimizing the results of various phonosurgeries.

KEY WORDS — elastic modulus, phonosurgery, vocal fold, Young's modulus.

INTRODUCTION

This paper describes a new device that measures Young's (elastic) modulus intraoperatively in the in vivo human vocal folds. Elastic modulus values may provide surgeons with an objective means of evaluating phonation intraoperatively, and therefore may be useful for guiding and optimizing results of phonosurgery. Traditionally, surgeons have gauged changes made to the vocal folds by visual inspection or by listening to the patient's voice. These judgments are purely subjective, and as a result surgical outcomes are often unpredictable. Because poor outcomes most often result from misjudgment of the appropriate amount of augmentation or medialization of the vocal folds, resulting in abnormal stiffening of the vocal folds, an objective procedure for evaluating vocal fold stiffness intraoperatively might improve the success rate for such surgeries, and would have broad application over a wide range of phonosurgical procedures.

Young's (elastic) modulus accurately represents the tissue elastic properties,[1] and was therefore chosen as a measurement standard. Young's modulus is calculated from the following equation: Young's modulus = Stress/Strain, where Stress is defined as force required for lateral movement, divided by tissue contact area over which the force is applied; and Strain is defined as change in length of x at a particular x, or amount of displacement. An alternate formulation for differential Young's modulus is ΔYoung's modulus = ΔStress/Strain, where ΔStress is differential stress, or the change in force divided by the tissue contact area over which the force is applied. This modulus measures both the physiologic and dynamic aspects of vocal fold compliance to variations in force and stimulation, and thus is probably a superior measure of fold properties[2] (also K. S. Stevens, unpublished observations).

Physiologically, the elastic modulus of the vocal fold tissue may be a prime determinant of the wave motion of the mucosal membrane.[3-6] The mucosal wave travels from the inferior portion of the folds to the superior, and then moves laterally onto their surface. This process transforms the egressing airstream through vocal fold vibration during phonation. The periodic interruption in airflow in turn produces the acoustic signal that is perceived as voice.[7] Hence, the elasticity of the vocal fold tissue is a key factor in the control of phonation.[8,9]

Anatomically, the layers of the vocal folds exhibit different mechanical properties.[10] Hirano[11] described these properties in the body-cover theory of vocal fold vibration. He described the vocal fold as a 2-layer system: a muscular layer (body) and a nonmuscular layer (cover). The cover consists of squamous epithelium and intermediate layers of loose connective tissue (lamina propria). It is very pliable, but has no intrinsic contractile property. The body includes the deep layer of the lamina propria and the vocalis muscle, whose contraction stiffens the body. These properties may contribute to the formation and characteristics of the traveling wave and the resulting

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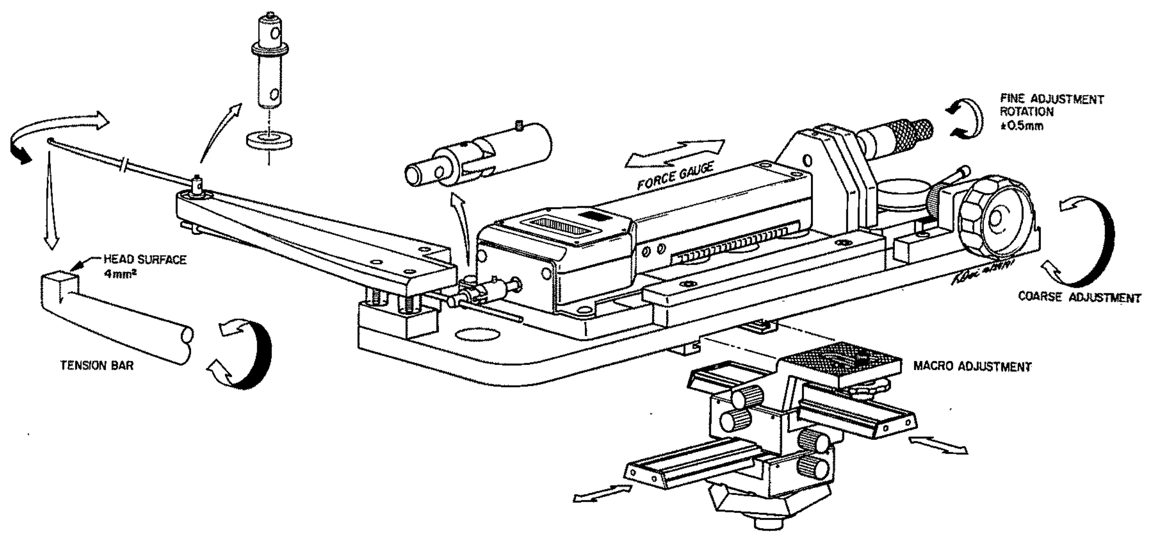

Fig 1. Diagram of new device for measuring differential Young's (elastic) modulus.

vocal qualities that can be produced.

Since the cover assumes most of the vibration near its surface,[12] an understanding of its elastic properties is of primary importance. Intuitively, it would seem that the combined stiffness of the entire vocal fold would be determined by the extrinsic longitudinal tension of the cover and the internal stiffening of the body. Thus, the elastic properties of both layers influence the vibration of the vocal folds.[13]

In vivo elastic modulus values have not been reported previously. However, a number of authors have measured in vitro elastic modulus values in order to predict the in vivo state of vocal fold vibration. Ishizaka and Kaneko[14] fastened a thread to a freshly cut human vocal fold, and then stretched the cord laterally to measure the displacement and force applied. From this preparation, they estimated the stiffness constant K at 3.7×10^4 dynes/cm. Kakita et al[2] investigated the mechanical properties of vocal fold tissues and reported ranges and orders of magnitude for Young's moduli and shear moduli of different layers of canine vocal folds. Weights were hung on the tissue samples, and the resulting elongation was measured with a microscope. Although their results provided some of the first quantitative estimates of the elastic moduli, methodological problems (including undefined reference length and questionable viability of the tissue) limit the generality of their measurements.

Perlman et al[15,16] investigated the in vitro elastic moduli of canine vocal fold tissues in viable condition. Longitudinal stepwise elongation was applied to the vocal folds, and the force was measured. From the force elongation curves and stress-strain curves of the vocal fold tissue, a secant Young's modulus for the body and cover was obtained. The longitudinal secant Young's modulus, defined as the ratio of total stress to the total increase in strain from a reference length, ranged from 1.0×10^6 dynes/cm^2 at 10% strain to 4.0×10^6 dynes/cm^2 at 50% strain.

Recently, Alipour-Haghighi and Titze[17] measured the in vitro longitudinal elastic modulus of canine vocal folds that were kept viable in Krebs solution. They used a slow cyclic stretch-and-release technique to measure the stress-strain in the range of 0% to 40% elongation over an approximate in situ length. These stress-strain data, which apply to longitudinal vocal fold tension, were modeled with polynomial and exponential functions. Alipour-Haghighi and Titze proposed that the elastic properties of the vocal fold tissues are nonlinear for both body and cover over most of the strain range, but that linear approximations can be made at low strain (strain < 15%). At these low strains, the cover has about twice the stiffness of the body as estimated with the longitudinal Young's modulus. Such low strain values are of interest, since they represent most normal phonation.[18]

Although no in vivo modulus measures have been reported, associated measures of vocal fold function made intraoperatively have been described. Fukuda et al[19] developed a technique in which an external vibrator was used to create a wave motion in the vocal folds. This motion was evaluated intraoperatively for symmetry by stroboscopy. However, the relationship between externally induced and natural vocal fold vibration is unclear. LeJeune[20] designed a device to measure the degree of vocal ligament tightening during laryngeal framework surgery. However, his device measured only strain, not stress. Further, strain was determined by pinching the vocal fold, possibly injuring the tissue. Berke et al[21] intraopera-

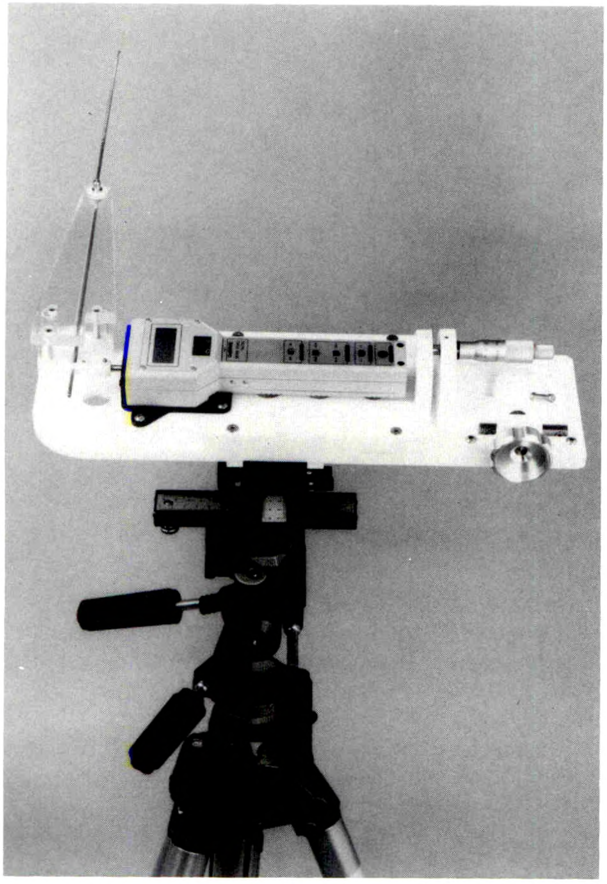

Fig 2. Photograph of actual device for measuring differential Young's (elastic) modulus.

tively monitored human vocal fold vibration by insufflating air rostrally through the vocal folds while manually adducting the arytenoids. This technique was used intraoperatively to monitor fold function and determine the end point for injecting Teflon into the vocal folds of a patient with previous failed attempts. However, the technique is imprecise and indirect compared to the direct stimulation of the laryngeal muscles.

The device described below is designed to overcome the limitations of previous methods by permitting measurement of vocal fold function in vivo. The device is described in detail below, along with its use in patients. We also report measurements of the activated muscular transverse elastic modulus.

METHODS

Device Construction. The device constructed to measure the Young's modulus of the in vivo human vocal folds is shown in Figs 1 and 2. This device was designed to meet several criteria. First, it moves into position easily and rapidly during phonosurgery, so that the elastic modulus can be measured quickly and easily. The device's deflection bar occupies very little space within the introitus of the larynx, and does not interfere with the surgical manipulation of the vocal fold. This allows dynamic measurement of the elastic modulus throughout a phonosurgical procedure.

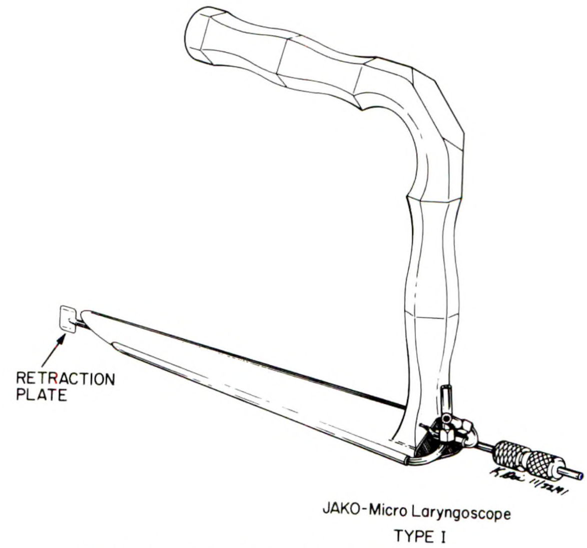

Fig 3. Diagram of modified Jako laryngoscope.

Second, the device measures the vocal fold's elastic modulus rapidly by deflecting the vocal fold a small distance, with measured force applied to a small area of the fold. Rapid measurement minimizes the effect of strain "creep" and stress "relaxation" in vocal fold tissue.[2] If uncontrolled, these factors will distort Young's modulus values and result in inaccurate measurements of the elasticity of the tissue. In our preliminary elastic modulus studies, stress "relaxation" was observed when force was applied to the tissue for longer than 30 seconds.

Third, the device is sensitive to very small changes in the modulus. The device incorporates a Shimpo Digital Force gauge (Shimpo force gauge DF-0.5R, Shimpo American Corp), which combines a light beam detection design with a microcomputer for measurement of push-pull forces. This gauge can measure forces from any angle and has a resolution of 0.1 g. This enables the device to record a change in Young's modulus of as little as 2,500 dynes/cm^2 per 0.2 mm of displacement. The computer interface ensures data can be processed and evaluated statistically during surgery.

This force gauge was placed on a lightweight platform that permits frictionless movement of the gauge during the measurement process. To facilitate the device's maneuverability and precise movement within the glottic area, coarse, intermediate, and micrometer adjustments are possible (Fig 1). The micrometer tuning adjustment produces precise, small

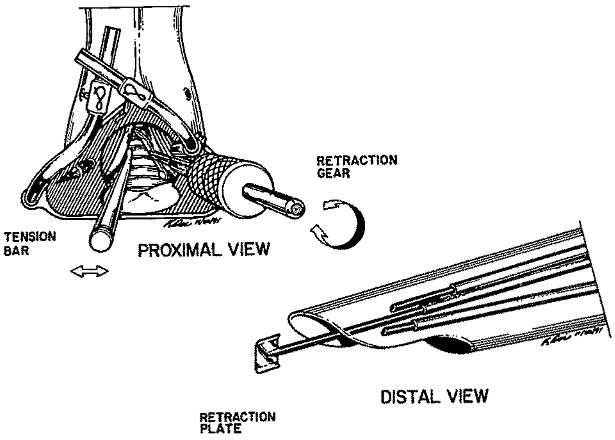

Fig 4. Placement of modified Jako laryngoscope within glottic area, showing footplate on stimulated vocal fold and retraction plate on nonstimulated vocal fold.

movements (as little as 0.1 mm) of the tissue contact footplate.

The deflection bar pivots at its midsection, so that any deflection or force exerted on the footplate by the stimulated vocal fold will result in an equal displacement or force on the gauge. A mechanical linkage between the deflection bar and the gauge facilitates smooth transmission of force, and also minimizes loss of force from the footplate to the gauge.

Measurement Procedure. Figures 3 and 4 show a Jako direct laryngoscope, which was modified for this study by incorporating a small retraction plate with a long handlebar within the laryngoscope. This plate is used to retract the nonstimulated vocal fold away from the deflection footplate. This prevents the nonstimulated vocal fold from touching the deflection footplate or bar. Any contact between the nonstimulated vocal fold and the device results in mismeasurement of the elastic modulus.

The modified Jako laryngoscope was suspended during the measurement (Fig 5). Use of a suspension laryngoscope stabilizes the thyroid cartilage and prevents unnecessary movement of the larynx. Pilot studies have shown that laryngeal movement interferes with elastic modulus measurement.

A small footplate is located at the end of the deflection bar. This plate has a surface contact area of 0.04 cm^2, and is placed posterior to the midsection of the vocal folds (Figs 4 and 5). This location reflects the greatest lateral excursion of the vocal folds during phonation, and thus is most representative of the fold's elastic property.[22] As the recurrent laryngeal nerve is electrically stimulated with a constant current, the affected vocal fold deflects the footplate medially and exerts a force on the force gauge.

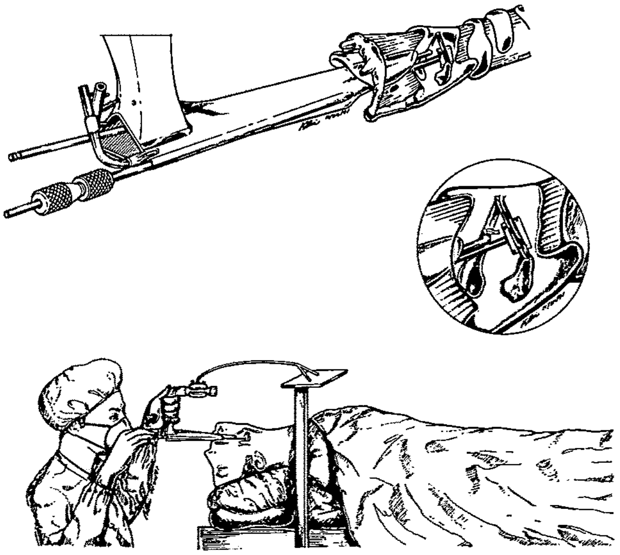

Fig 5. Diagram of modified Jako laryngoscope during its suspension and stabilization of glottis.

At the beginning of each measurement, the device must be initialized by using the intermediate and micrometer tunings to adjust the footplate, so that the force exerted on the footplate by the vocal fold is zero. The resulting position of the footplate at zero force is defined as the initial zero displacement.

In Vivo Human Study. Five men volunteered for this study. They ranged in age from 40 to 50. Four of the 5 subjects had no history of laryngeal lesions or voice problems, and preoperative indirect laryngoscopy and thorough head and neck examination revealed no current laryngeal or voice disorder. One subject had evidence of a hyperfunctional voice disorder, characterized by a moderately rough voice and no laryngeal lesion.

After appropriate research protocol consents were obtained, the subjects were brought to the operating room and placed supine on the operating table. Five milliliters of 1% Xylocaine was used to infiltrate the sensory branch of both superior laryngeal nerves to suppress potential laryngospasm. A slow mask ventilation with 3.5% isoflurane–nitrous oxide and 100% oxygen supplement was used until the subject's depth of anesthesia was maximized. Subsequently, the subject underwent orotracheal intubation with an endotracheal tube (No. 5). Thereafter, 2% isoflurane gas was used to maintain anesthesia during the measurement process. No paralytic agent was used during the study.

The subject's neck was gently hyperextended, draped, and prepared for transcutaneous recurrent laryngeal nerve stimulation. This technique has been described previously and used relatively safely in humans.[23] Two monopolar Teflon-coated needles were inserted through the skin. One was inserted

TABLE 1. MEASURED YOUNG'S MODULUS VALUES FOR THREE STIMULATION LEVELS

	Rest	*High Stimulation*	*Low Stimulation*
Mean	126,616	215,894	191,688
SD	76,349	11,432	111,854
Minimum	24,500	58,800	41,650
Maximum	294,000	441,000	416,500

Data are dynes per square centimeter.

down to the level of the posterior aspect of the left cricoid cartilage, and 1 was inserted along the left tracheoesophageal groove inferior to the cricoid cartilage. These needles conducted various currents from a nerve stimulator (model S2LH, WR Medical Electronics Co, St Paul, Minn) to stimulate the left recurrent laryngeal nerve. The nerve was transcutaneously stimulated at 80 Hz, with 2 different currents (3.8 mA and 3.2 mA); pulse duration was 1.5 milliseconds.

All measurements were made in the subject's left vocal fold. Three separate measurements of the elastic modulus were made: in the fold's resting state; at a high stimulation level, at which point the left vocal fold adducted to midline with prominent vocalis bulging (3.8 mA); and at a lower stimulation level (3.2 mA).

All subjects received 3 blocks of trials (1 per stimulation condition). The resting condition was always first, followed by the high stimulation condition and then the low stimulation condition. A rest period of 3 to 5 minutes separated adjacent stimulation conditions to reduce possible effects of vocalis muscle fatigue. Our previous experience with the in vivo canine model indicates that this period is sufficient.

Within each condition, 2 complete measurement cycles were made in rapid succession. At the beginning of the experiment, the footplate was placed posterior to the midsection of the vocal fold, and initialized as described above. The footplate remained in contact with the vocal fold until the entire experiment was completed. The appropriate stimulation was applied, displacement was increased in 0.5-mm steps from 0 to 3.0 mm, and force was measured for each displacement level. The device was then reset to zero, and the sequence of measurement was repeated. The 2 sets of measurements within a condition lasted less than 25 seconds.

At each increment, the vocal fold exerted different forces on the footplate. These forces reflect the inherent elastic property of the tissue at that given tissue displacement (stretch). Force values were recorded and processed by the computer to derive values of Young's modulus as follows. First, the measured mass at each displacement level was multiplied by the constant 980 cm/s^2 to derive force (mass multiplied by acceleration). Stress was then calculated by dividing the force at each displacement by the area of the footplate (0.04 cm^2). Next, differential stress (ΔStress) was calculated by using the formula $(F - F_0)/A$, where F is force at the current displacement, F_0 is force at the previous displacement, and A is the area of the footplate. Similarly, strain was calculated as $(x - x_i)/x_i$, where x is the current displacement and x_i is the previous displacement. The differential Young's modulus is the ratio of differential stress to strain, or ΔStress divided by Strain.

RESULTS

To determine how well in vivo measurements of Young's modulus could be reproduced, we examined the correlation between the first and second measurements at each displacement and stimulation level, for the group as a whole and within each subject. The different stimulation conditions were combined for this analysis. For the group as a whole, Pearson's r was .84; values for individual subjects ranged from .97 to .79 (all correlations significant at $p < .05$). These values indicate very good measurement replicability, and suggest that the device may be used reliably to measure vocal fold elasticity in vivo.

Table 1 gives the mean, standard deviation, and ranges of Young's modulus values for the 3 stimulation conditions. Measurements are in dynes per square centimeter. A 2-way (stimulation condition by subject) analysis of variance using the differential Young's modulus as the dependent variable showed significant differences among stimulation conditions ($F_{2,135} = 9.42$, $p < .05$), no significant differences between subjects ($F_{4,135} = 0.09$, $p > .05$), and no significant interaction between subjects and conditions ($F_{8,135} = 0.08$, $p > .05$). Post-hoc Scheffé comparisons showed that the resting condition differed significantly from high stimulation ($p < .01$) and from low stimulation ($p < .01$); the high and low stimulation conditions did not differ significantly from one another ($p = .53$).

Figure 6 shows the relationship between the differential Young's modulus and vocal fold displacement. Young's modulus increased in a linear fashion with displacement, for all stimulation conditions (for the resting state, $F_{1,53} = 532.12$, $p < .05$, $r^2 = .91$; for high stimulation, $F_{1,53} = 295.00$, $p < .05$, $r^2 = .85$; and for low stimulation, $F_{1,53} = 166.56$, $p < .05$, $r^2 = .76$).

DISCUSSION

Our results demonstrate that the new device pro-

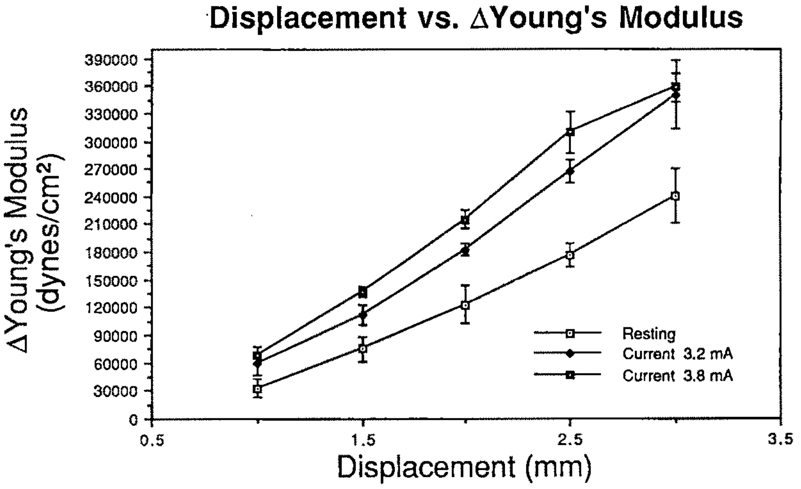

Fig 6. **Graph of vocal fold displacement versus differential Young's modulus.**

duces reliable in vivo measurements of the differential Young's modulus. The measurements did not differ significantly among speakers, suggesting that similar stimulation conditions will result in comparable changes in elasticity across individuals. Although our results must be considered preliminary, findings of consistent responses across subjects may make it possible to derive standardized target values for Teflon augmentation surgery. The availability of such values might improve the outcomes of phonosurgery.

In the present study, we found that Young's modulus increased in a linear fashion with vocal fold displacement, at all levels of stimulation. Across stimulation levels, the elastic modulus values for the vocal fold at rest differed significantly from those measured under transcutaneous stimulation, but the 2 different stimulation levels did not differ significantly from one another. There are several possible explanations for this finding. First, fatigue effects may be present. The stimulation levels were not randomized in this study; instead, all subjects received the different conditions in the same order (rest, high stimulation, and low stimulation). This strategy ensured that differences among subjects were not confounded with presentation order, adding credibility to our finding that subjects did not differ in modulus values at any stimulation level. However, Table 1 shows that modulus values were much more variable in the low stimulation condition (which occurred last) than under high stimulation. This suggests that subjects differed in the extent to which fatigue affected them. Had measurements in the low stimulation condition varied less, differences between the 2 stimulation conditions might have reached statistical significance.

It is also possible that stimulation levels did not differ sufficiently to produce reliable differences in the Young's modulus values. The stimulation levels were chosen by observing the effect of stimulation on the vocal folds: stimulation at 3.8 mA consistently produced adduction to midline with prominent vocalis bulging, while stimulation at 3.2 mA resulted in visible relaxation of the folds. However, the current actually delivered to the nerve via transcutaneous stimulation may have differed from that generated by the stimulator; further, ceiling effects may have been present, with 1 or both stimulation levels producing contractions near the fold's physiologic limit. Our ability to separate these factors is limited in the in vivo model. However, further studies controlling for presentation order effects are clearly indicated.

This study represents the first attempt to measure Young's modulus in vivo. In the present study, the in vivo transverse elastic properties of body and cover tissues were reported as 1 entity. Because tissue exists in 3 dimensions, it potentially has different longitudinal, vertical, and transverse elastic moduli.[2,4,24,25] Previous studies have generally measured the elastic modulus in the longitudinal plane (anterior-posterior direction), which is the most isotropic. However, this modulus cannot be measured in the in vivo state because of the anterior and posterior attachments of the vocal folds. The transverse modulus can be measured in vivo without harm to the subject. Further, the differential transverse modulus is a dynamic measurement, and reflects the change in the vocal fold elasticity at any given tissue displacement. Finally, most of the vocal fold tissue wave movement is in the transverse direction (although the traveling wave motion in the vocal fold is in the vertical dimension). These factors combine to make the transverse modulus a logical choice for measurement in vivo.

Moduli reported in this study were much smaller than the values of the longitudinal modulus reported previously by Kakita et al.[2] Our values also differ from those of Alipour-Haghighi and Titze,[17] who

TABLE 2. ESTIMATION OF WAVE VELOCITY VALUES USING CALCULATED DIFFERENTIAL YOUNG'S MODULUS

	Rest	*High Stimulation*	*Low Stimulation*
Mean	338.1	446.8	418.3
SD	112.0	129.0	130.6
Minimum	156.5	242.5	204.1
Maximum	542.2	664.1	645.4

Data are centimeters per second.

reported longitudinal Young's moduli for the body and cover of 20.7 kilopascals and 41.9 kilopascals, respectively, at low strain (0% to 15%). These values are at least 4 to 5 times higher than our measurements, regardless of the stimulation current on the affected vocal fold. These differences were probably due to the sensitivity of our device, the choice of Young's elastic modulus, and the in vivo technique of measurement. That the transverse moduli also increased linearly with displacement suggests a low strain range (strain < 15%) of vocal fold tissue deformation. This may be significant in that the vocal folds operate mostly at low strains during phonation.[18]

This study measured Young's modulus in subjects under general anesthesia. Many phonosurgical techniques are currently performed under local anesthesia in awake patients. This device is not practically suited to such operations. A noninvasive technique to estimate the stiffness of the vocal folds should certainly be the subject of future investigation. The data from the present study provide valuable norms to which results of future studies may be compared. Toward this end, it is important to emphasize that measurement of Young's modulus values should permit the calculation of traveling wave velocity by the formula Traveling wave velocity = (Young's modulus/tissue density)$^{1/2}$.

The traveling wave velocities calculated from our measurements are given in Table 2. The traveling wave velocity has been observed to change in some laryngeal disorders that affect vocal fold stiffness, such as unilateral recurrent laryngeal nerve paralysis.[26] A method of measuring wave velocity in vivo has obvious implications for the diagnosis and treatment of such disorders.

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4TH INTERNATIONAL ACADEMIC CONFERENCE IMMUNOBIOLOGY IN OTOLOGY, RHINOLOGY AND LARYNGOLOGY

The 4th international academic conference Immunobiology in Otology, Rhinology and Laryngology will be held in Oita, Japan, April 4-7, 1994. For further information contact Goro Mogi, MD, Dept of Otolaryngology, Oita Medical University, Hasama-machi, Oita 879-55, Japan; telephone 81-975-49-4411 ext 2912 or 2913; fax 81-975-49-0762.

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CARCINOMA IN SITU OF THE GLOTTIS: RADIOTHERAPY OR EXCISION BIOPSY?

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Excision biopsy, radiotherapy, and laryngofissure cordectomy have all been used, but the best treatment regimen for glottic carcinoma in situ remains controversial. A 20-year experience of 37 cases is reported. For the first 10 years biopsy with radiotherapy was the first-line treatment (n = 20), but for the last 10 years surgical or laser excision biopsy alone has been performed (n = 17). The T stage of the carcinoma in situ does not determine its premalignant potential. Malignancy appears to be a recurrence, rather than a new expression of carcinogenesis. Excision biopsy alone provides local control results (75%) comparable to those of primary radiotherapy (85%). Excision biopsy alone, however, retains the option of subsequent radiotherapy with preservation of laryngeal function, but very close follow-up, especially in the first year, is required.

KEY WORDS — carcinoma in situ, glottis, radiotherapy.

INTRODUCTION

Although Chevalier Jackson in 1923[1] first recognized a possible link between dysplasia of the larynx and carcinoma, it is Broders in 1932[2] who is credited with the first description of carcinoma in situ of the larynx. It is a replacement of the normal histologic structure of squamous epithelium by cells of a malignant morphological pattern, but the basement remains intact. It is represented by "Tis" under the UICC classification of laryngeal cancer.[3] The reported incidence of carcinoma in situ without coexisting invasive carcinoma varies widely, from 1%[4] to 16%[5] of laryngeal malignancies.

Following the introduction of the microlaryngeal surgical technique by Kleinsasser[6] in 1968 and the carbon dioxide (CO2) surgical laser by Strong[7] in 1974, laryngofissure cordectomy as a form of surgical treatment of carcinoma in situ fell into disrepute. Radiotherapy, however, continued to be used widely. In a 1981 survey of 114 otolaryngologists, Ferlito et al[4] identified a wide international variation in treatment policy. Despite numerous reports over the last 20 years,[3,8-19] the optimum management of this condition remains unclear. Why is this so?

Authors have not always restricted their reports to carcinomas in situ. It is generally accepted that the greater the degree of dysplasia, the greater the likelihood of progression to malignant invasion.[20] The inclusion of cases of dysplasia or even microinvasion through the basement membrane (T1), therefore, distorts the results.

Even reports confined to carcinoma in situ have not always described the same condition. Despite the proposals of Kleinsasser,[21] The Centennial Conference on Laryngeal Carcinoma–Toronto,[22] and Hellquist et al,[13] there is still no universal grading of dysplasias and their separation from carcinoma in situ. It is therefore little surprise that results vary.

A randomized controlled trial has yet to be reported. The majority of the literature describes results from 1 treatment modality only. In the few in which more than 1 modality was used, the criteria for determining which modality was used were variable. We report our experience over the last 20 years (1972 to 1991), in which radiotherapy was the treatment of choice during the first decade but surgery was the first-line therapy thereafter.

MATERIALS AND METHODS

The histology and clinical records of 63 patients with dysplastic lesions of the glottis treated in the Departments of Otolaryngology and Radiotherapy, General Hospital, and the Department of Otolaryngology, University Hospital, in Nottingham from

TABLE 1. HISTOPATHOLOGIC CLASSIFICATION OF PREMALIGNANT LARYNGEAL LESIONS

Group	*Definition*
I	Squamous cell hyperplasia with or without keratosis and/or mild dysplasia
II	Squamous cell hyperplasia with moderate dysplasia
III	Squamous cell hyperplasia with severe dysplasia or classic carcinoma in situ with full-thickness atypia

Information from Hellquist et al.[13]

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 11-12, 1992.

TABLE 2. CASES OF GLOTTIC CARCINOMA FOLLOWING TREATMENT

Pt	*Age at Diagnosis*	*Stage*	*VC*	*First Treatment*	*Interval (mo)*	*Stage*	*VC*	*Second Treatment*	*Present Status*
1	65	T1a	R	XRT	19	T2N0	R*	TL	NED at 72 mo
2	50	T1b	Both	XRT	42	T4N0	R†	TL	Died of lung primary at 16 mo
3	45	T1a	R	XRT	193	T3N0	R	TL	NED at 10 mo
4	62	T1a	R	XB	3	T1	R	Rx	NED at 72 mo
5	48	T1a	L	XB	7	T1	L	Rx	NED at 60 mo
6	76	T1a	L	XB	17	T1	L	Rx	Alive at 108 mo
7	59	T1a	R	XB	7	T1	R	Rx	NED at 31 mo
8	62	T1a	R	XB	11	T3	R	TL	Died of local disease at 34 mo

"Stage" is that of UICC system for invasive squamous cell carcinoma.

VC — vocal cord, XRT — primary irradiation, XB — excision biopsy alone, TL — total laryngectomy, Rx — radiotherapy, NED — no evidence of disease.

*Glottis and supraglottis.

†Anterior commissure and VC.

1972 through 1991 were reviewed. Thirty-seven had group III disease according to the histopathologic classification of premalignant lesions proposed by Hellquist et al[13] (Table 1). It is these that form the basis of this report.

The mean age was 63.4 years (range 39 to 91). There were 29 men and 8 women, of whom 27 admitted to being smokers at the time of diagnosis. According to the same UICC staging system used for invasive squamous cell carcinoma of the glottis, 29 had T1a disease, 7 had T1b, and 1 had T2.

From 1972 through 1981 the primary treatment was biopsy with radiotherapy. Twenty patients received 60 Gy over 25 fractions from megavoltage equipment using a cobalt 60 unit of a 6-megavolt linear accelerator. Mean follow-up time was 14.4 years. From 1982 the primary treatment has been excision biopsy alone. From 1982 to 1987 this was performed with microcupped forceps on 14 patients, and from 1988 to 1991, 3 patients were treated with a Coherent 451 CO2 laser (Coherent Medical Division, Palo Alto, Calif). Mean follow-up time was 5.1 years; minimum follow-up was 15 months.

RESULTS

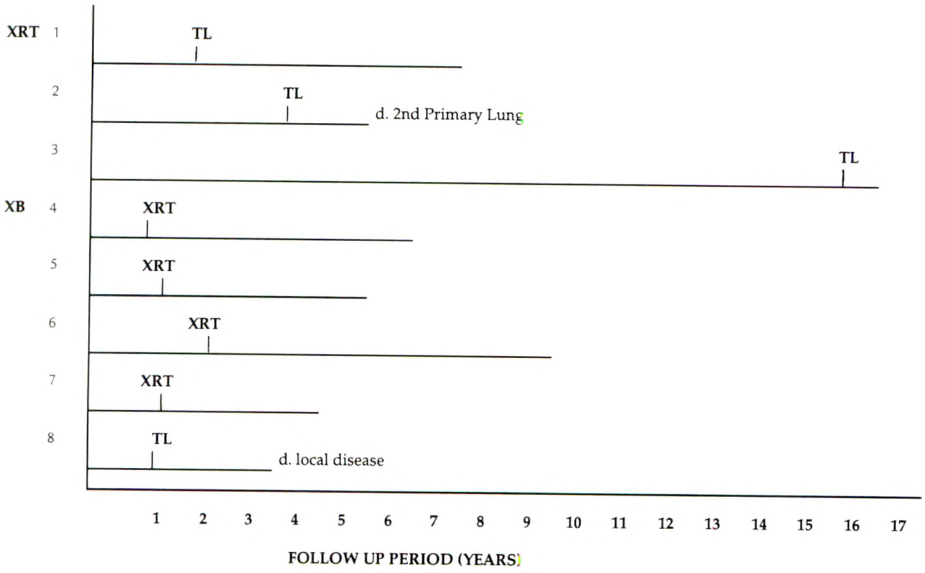
The mean age of the 20 patients receiving biopsy with radiotherapy was 63 years (39 to 91). Fifteen were men, and 19 of the 20 admitted to being smokers at the time of diagnosis. Local control was achieved in 85% (17 of 20) of patients. Of these, 10 are alive with no evidence of disease and 7 are dead from unrelated causes, including 1 from a lung primary. Three patients developed glottic carcinoma, the details of which are summarized in Table 2 (patients 1 through 3). Two of the 16 patients with stage T1a cancer, and 1 of the 3 patients with T1b, developed glottic carcinoma. The 1 patient with T2 cancer is disease free.

The mean age of the patients treated with excision biopsy alone was 63.8 years (49 to 81). Fourteen had the procedure performed with microcupped forceps, and 3 with laser. Fourteen were men, and 8 admitted to being smokers at the time of diagnosis. Local control was achieved in 71% (12 of 17) of patients. Of these, 11 are alive with no evidence of disease, and 1 died of a second primary in the lung. The details of the 5 patients developing glottic carcinoma are summarized in Table 2 (patients 4 through 8). None of the patients treated with laser therapy has yet developed carcinoma. Patient 8 initially presented with a squamous cell carcinoma of the floor of the mouth, which was surgically treated. The glottic carcinoma in situ developed 29 months later. Of the 13 patients with T1a disease, 5 developed glottic carcinoma. None of the 4 patients with T1b disease underwent malignant change.

The Figure compares those patients who developed glottic carcinoma following biopsy and radiotherapy with those who developed it following excision biopsy alone.

DISCUSSION

At first sight the primary use of adjunctive radiotherapy appears to provide better local control (85%) than excision biopsy alone (71%). The fifth case of glottic carcinoma in the excision biopsy alone group is, however, atypical in that it is the only one of 37 cases that had a preexisting head and neck primary cancer, and although death occurred from local head and neck disease, it was not possible to determine whether this was attributable to the floor of mouth cancer or the laryngeal cancer. Excluding this case increases local control using excision biopsy alone



Glottic carcinoma in situ developing carcinoma after treatment with primary radiotherapy (XRT) or excision biopsy alone (XB). TL— total laryngectomy.

from 71% to 75% (12 of 16).

A comparison of the time interval between diagnosis of carcinoma in situ and the appearance of invasive carcinoma reveals that malignant change occurred significantly earlier in the group undergoing excision biopsy alone. That 3 cases of malignancy were identified within the first 8 months raises the question of whether malignant change had indeed occurred or whether the original biopsy was unrepresentative. Clear margins were present, however, in the original biopsies of all 3 cases. Close follow-up, particularly in the first year, is therefore essential.

A link between the T stage of the carcinoma in situ and its potential to undergo malignant change has been postulated. Seven of the 8 cases that became carcinoma were T1a, and the eighth was T1b. The single case staged T2 did not become invasive carcinoma. The evidence reported here, therefore, does not support such a theory.

In all 8 cases in which carcinoma developed, the site and side involved corresponded to those of the carcinoma in situ; this finding suggests a recurrence rather than a new expression of carcinogenesis.

CONCLUSION

Excision biopsy alone for glottic carcinoma in situ provides local control results comparable to those obtained with biopsy with radiotherapy. Excision biopsy alone, however, retains the option of subsequent radiotherapy with preservation of laryngeal function. If excision biopsy alone is the treatment of choice, very close follow-up, especially in the first year, is required. There is no evidence to suggest that the T stage of carcinoma in situ determines premalignant potential. Where malignant change does occur, it appears to be a recurrence rather than a new expression of carcinogenesis. Laser treatment may prove to be superior to microcupped forceps stripping, but further data are awaited.

ACKNOWLEDGMENT — We thank Kathryn Vickers for her advice in the preparation of this manuscript.

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SIXTH INTERNATIONAL CONGRESS ON INTERVENTIONAL ULTRASOUND

The Sixth International Congress on Interventional Ultrasound will be held September 7-10, 1993, in Copenhagen, Denmark. For information, contact Christian Nolsøe, Congress Secretary, Department of Ultrasound, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark.

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FIRST CONGRESS OF EUROPEAN SKULL BASE SOCIETY

The European Skull Base Society will hold its first congress September 26-30, 1993, in Riva del Garda (Trento), Italy. For further information contact Carmelo Zappone, MD, Congress Secretary, Division of ORL, Ospedali Riuniti, Largo Barozzi 1, 24100 Bergamo, Italy; telephone 35-269578; fax 35-269460.

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EXPERIMENTAL ENDOLYMPHATIC HYDROPS AND GLYCEROL

ELECTROPHYSIOLOGIC STUDY

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The object of the present study was to evaluate electrophysiologically the therapeutic effect of glycerol on the cochlear function of guinea pigs in which experimental hydrops had been surgically induced. Fifteen albino guinea pigs were used. Each animal was chronically implanted to follow the evolution of hearing. The experimental protocol considered 3 groups of guinea pigs (5 animals each). Group 1 received no drug treatment, while groups 2 and 3 were given glycerol orally at 0.75 and 0.50 g/kg of body weight once a day for 4 months. The audiograms of the animals treated with glycerol showed a statistically significant hearing improvement compared with the control group. Clinically, our results seem to support the view of those who favor the use of glycerol for the medical treatment of Meniere's disease at an early stage.

KEY WORDS — electrocochleography, endolymphatic hydrops, glycerol.

INTRODUCTION

In Meniere's disease, glycerol has been widely employed as a diagnostic aid.[1,2] This osmotic agent also has been used by De Vincentiis et al[3,4] for medical treatment of many patients in the initial stages of the disease; they have documented successful outcomes with prolonged hearing improvements. We recently investigated the histologic effect of medical treatment with glycerol in the guinea pig model of experimental hydrops.[5-8] The purpose of the present study was to explore electrophysiologically the therapeutic effects of glycerol on the cochlear function of guinea pigs in which experimental endolymphatic hydrops had been surgically induced. In order to determine the optimum dose of glycerol required to prevent hearing loss, we carried out a trial of treatment with 0.5 g/kg and 0.75 g/kg of glycerol, which in our former histologic studies[5-8] represented the optimal dosages in controlling the extent of the hydrops.

MATERIAL AND METHODS

Fifteen albino guinea pigs of both sexes (weighing 450 to 550 g each) were used in this study. All animals had normal pinna reflex thresholds and were free of middle ear infection as judged by direct observation.

The animals were anesthetized with diazepam (0.25 mg/kg) and ketamine hydrochloride (40 mg/kg) prior to chronic implantation. According to the technique proposed by Hildesheimer et al,[9] the active electrode (stainless steel wire) was introduced 4 mm into the tight facial canal through the stylomastoid foramen. This electrode and the reference and ground electrodes were fitted with a small connector, which was fixed to the skull with dental cement. One week after implantation, the guinea pigs were again anesthetized, and ipsilateral obliteration of the endolymphatic sac and duct was performed with an extradural posterior fossa approach, as described in detail elsewhere.[10]

The experimental protocol considered 3 groups of guinea pigs (5 animals each). Group 1 (control group) received no drug treatment. In the other groups glycerol was given at dosages of 0.75 g/kg (group 2) and 0.50 g/kg (group 3) orally once a day beginning the first postoperative week after obliteration. The drug was administered for the first 3 months. During the fourth month, the animals received no glycerol and the drug administration was started again in the fifth month, to evaluate the duration of the effect of the medical treatment.

All of the recordings of the compound action potential (CAP) audiogram were registered in an electrically shielded, soundproof room; air-conducted acoustic stimuli were delivered through a TDH-3 earphone, as suggested by Bohmer.[11] The acoustic stimuli consisted of tone pips of 1, 2, 4, and 8 kHz (four cycles; 4 milliseconds' duration for 1 kHz, 0.5 milliseconds for 8 kHz with a sinusoidal envelope). The stimuli were presented 400 times, and the electrical responses were filtered and averaged by a Medelec digital average system.

The recording sessions were performed before obliteration of the endolymphatic sac and duct, 8 days after the surgically induced hydrops, and then every

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COMPOUND ACTION POTENTIAL THRESHOLDS TO RANGE OF STIMULUS FREQUENCIES

Day	*1,000 Hz*	*2,000 Hz*	*4,000 Hz*	*8,000 Hz*
Group 1 (control)				
8	23.3 ± 2.3	21.3 ± 3.5	19.8 ± 4.7	3.3 ± 0.9
15	27.8 ± 4.4	23.6 ± 3.8	23.7 ± 2.5	10.1 ± 2.7
30	28.3 ± 5.7	29.3 ± 6.5	30.2 ± 3.9	10.0 ± 2.2
45	29.1 ± 4.9	35.7 ± 7.1	39.5 ± 5.2	10.7 ± 3.5
60	35.0 ± 6.3	39.6 ± 4.9	41.6 ± 5.9	13.3 ± 2.9
75	48.0 ± 7.1	44.3 ± 5.9	42.3 ± 6.1	27.2 ± 3.3
90	53.3 ± 4.8	53.3 ± 7.8	40.0 ± 6.1	33.3 ± 4.7
105	53.6 ± 5.1	54.1 ± 6.9	52.4 ± 7.3	34.7 ± 3.9
120	55.0 ± 7.1	55.0 ± 7.6	52.5 ± 8.2	35.0 ± 4.9
Group 2 (0.75 g/kg)				
8	20.3 ± 1.9	19.1 ± 3.1	17.7 ± 3.9	5.1 ± 0.3
15	17.6 ± 2.3	9.8 ± 1.7	14.3 ± 4.1	3.3 ± 0.3
30	11.7 ± 2.1	9.3 ± 1.9	27.9 ± 3.4	0.0 ± 0.5
45	14.8 ± 2.7	9.7 ± 1.5	20.1 ± 2.9	0.0 ± 0.5
60	15.0 ± 1.9	13.3 ± 2.1	30.1 ± 3.1	0.0 ± 0.7
75	15.0 ± 1.5	14.7 ± 2.2	30.0 ± 1.9	0.0 ± 0.9
90	15.0 ± 1.7	16.8 ± 3.5	30.0 ± 2.9	5.0 ± 1.2
105	23.1 ± 2.3	24.3 ± 3.9	37.8 ± 4.1	10.3 ± 1.7
120	35.0 ± 4.3	31.8 ± 2.9	47.5 ± 7.1	15.0 ± 2.5
135	29.9 ± 4.6	23.7 ± 2.8	31.5 ± 6.9	12.7 ± 2.3
150	25.0 ± 2.8	18.3 ± 2.7	17.5 ± 3.2	10.0 ± 2.3
Group 3 (0.50 g/kg)				
8	24.1 ± 4.1	20.3 ± 3.9	18.3 ± 4.2	2.5 ± 1.1
15	10.3 ± 2.1	17.1 ± 3.4	16.2 ± 3.5	2.5 ± 1.1
30	13.3 ± 2.6	9.3 ± 3.5	13.3 ± 4.5	3.3 ± 0.8
45	13.1 ± 2.9	13.1 ± 3.7	13.7 ± 4.1	3.3 ± 1.1
60	13.3 ± 2.9	13.3 ± 4.1	13.3 ± 3.8	0.0 ± 0.7
75	14.1 ± 2.1	13.7 ± 4.2	14.3 ± 4.2	0.0 ± 0.7
90	3.3 ± 0.9	13.3 ± 3.6	13.3 ± 5.1	3.3 ± 0.8
105	20.8 ± 5.3	29.3 ± 6.9	27.7 ± 7.1	16.8 ± 3.9
120	48.3 ± 5.9	53.3 ± 7.9	53.3 ± 8.9	28.3 ± 2.9
135	40.2 ± 3.7	37.4 ± 6.1	36.8 ± 8.6	15.5 ± 4.5
150	38.3 ± 5.7	23.3 ± 4.8	23.3 ± 3.7	3.3 ± 0.7

Data are mean ± SD in decibels sound pressure level.

15 days thereafter. Before each test session the animals were denied food, water, and glycerol for at least 12 hours.

Mean values and standard deviations of the CAP thresholds of each tested frequency were calculated for the total number of animals in each group. Analysis of variance was performed on data concerning mean values.

RESULTS

In guinea pigs without treatment (group 1) a progressive hearing loss was observed. The CAP threshold deteriorated selectively in the first 60 days for the frequencies 1,000, 2,000, and 4,000 Hz. The mean threshold shift, however, showed a diminution of action potential that increased with time (see Table).

In the recordings taken 8 days after surgical obliteration of the endolymphatic sac and duct, the audiometric findings of groups 2 and 3 did not differ from those of the control group. After the administration of glycerol the hearing improved (see Table). Such an improvement was observed at the 4 different frequencies throughout the observation period of 90 days. It was statistically significant compared with group 1 ($p < .01$).

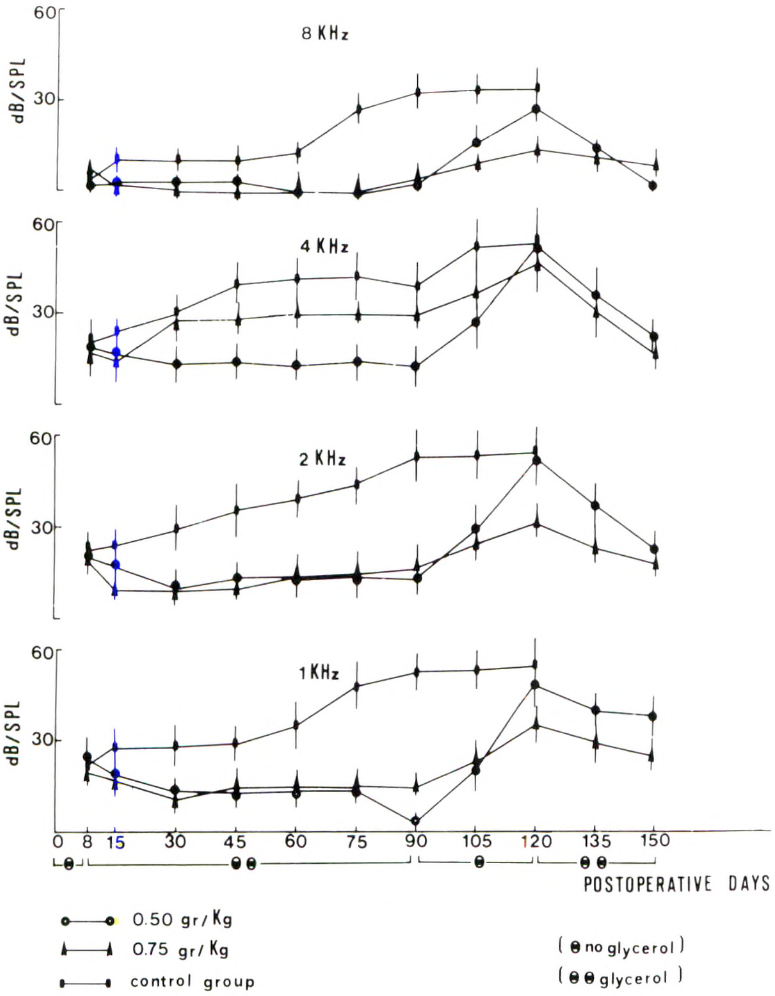
During the next month the guinea pigs received no glycerol. In this condition, the hearing loss progressed to about 15 to 47.5 and 28 to 53.3 dB, respectively, for groups 2 and 3. Very little difference was found from the control group, showing that the glycerol effect was strictly linked to its period of administration. With further medical treatment, there was a recovery of sensitivity across the audiogram. This was more pronounced for the animals that were given 0.75 g/kg, although the hearing did not reach the previous level.

The evolution of CAP threshold as a function of days after operation at 4 different frequencies is summarized in the Figure. Statistical analysis showed a significant difference ($p < .01$) in the hearing levels between the nontreated (group 1) and treated animals for all frequencies compared 30, 60, and 90 days after the induction of hydrops. No statistical difference ($p > .2$) was seen after 8 days between group 1 and groups 2 and 3. In addition, the difference between groups 2 and 1 after 120 days was not significant ($p > .4$). In contrast, the mean values of group 2 differed statistically from those of the other groups ($p < .01$), except for the frequency 4,000 Hz ($p > .21$).

DISCUSSION

In examining the present electrophysiologic data, 2 important findings deserve close analysis. First, the CAP threshold of the group without drug treatment displayed a pattern similar to that seen by other investigators.[12,13] The thresholds disclosed a progressive elevation only below 4 kHz in the early stage of the endolymphatic hydrops (60 days). The evolution of the hydropic status showed an involvement of the middle frequencies, which presented hearing loss after 3 months. These findings indicate that the hydropic animal model has many audiologic relationships with Meniere's disease in humans, confirming its experimental validity.[13-17]

The second observation was that a statistically significant improvement in hearing thresholds occurred in the 2 groups of guinea pigs treated with glycerol. This agent had similar ameliorating effects at both the 0.75 g/kg and 0.50 g/kg dosages, which represent 0.5 and 0.33 times the clinical dosage, respectively, for the "glycerol test" used in Meniere's disease, and prevented hearing fluctuation. Electro-



Evolution of compound action potential thresholds throughout follow-up period.

physiologic measures evidenced that CAP thresholds worsened when drug administration was suppressed. The hearing of group 3 (0.50 g/kg) reached analogous levels to those of the control group, while in group 2 (0.75 g/kg) the deterioration was less severe, showing a significant difference ($p < .1$) from the other groups. This outcome suggests that this dosage had a more pronounced effect in controlling the experimental hydrops. When glycerol was readministered, the audiologic deficit recovered, although the thresholds did not reach the former levels. The 0.75 g/kg dosage again gave evidence of better cochlear function, supporting the existence of a dosage-related effect. The present electrophysiologic data are well correlated with our previous histologic studies.[5-8] The daily administration of different dosages of glycerol, ranging from 2 g/kg to 0.5 g/kg for 1 month, markedly reduced the extent of the area of the scala media. It is interesting to note that after 0.5-g/kg glycerol treatment, minimal hydrops was found, while 0.75 g/kg completely prevented dilatation of the endolymphatic compartment.

Other investigators have examined glycerol's effect on surgically induced endolymphatic hydrops.[14-19] Horner and Cazals[16] found a deterioration of auditory function. Similar results were reported by Kusakari et al[15,19] and others.[16-18] The most plausible cause for these discrepancies with our experience is that in their experiments they tried to reproduce the equivalent of the glycerol test, while here the approach was therapeutic. Additional studies will be needed to investigate the therapeutic effect of glycerol on long-term hydrops of the animal ear.

Clinically, our results seem to support the view of those who favor glycerol for the medical treatment of Meniere's disease at an early stage. Minimal dosages (0.50 g/kg or 0.75 g/kg) are indicated.

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TWENTY-NINTH POSTGRADUATE COURSE IN EAR SURGERY

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EXPERIMENTAL OTITIS MEDIA WITH EFFUSION INDUCED BY PLATELET ACTIVATING FACTOR

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This study tested the hypothesis that platelet activating factor (PAF) in the middle ear can induce otitis media with effusion (OME) and that PAF antagonists can prevent PAF-induced OME. An initial trial of 16 μg of PAF was injected into chinchilla bullae, and all ears developed middle ear effusion (MEE) within 48 hours. Subsequent trials were performed to test dose dependency. Interestingly, 1 or 16 μg of PAF caused more MEE and inflammation than did 4 or 8 μg. A dose of 0.5 μg PAF did not cause MEE. Middle ear effusion from injected bullae contained the full spectrum of lipoxygenase and cyclooxygenase products; additionally, more PAF was detected than was injected. Finally, a PAF antagonist (WEB 2170) injected intraperitoneally prevented PAF-induced OME. This study demonstrates that PAF injected into the middle ear can induce OME and that PAF antagonists effectively prevent PAF-induced OME. These findings suggest that PAF plays an important role in the pathogenesis of OME.

KEY WORDS — otitis media with effusion, PAF antagonist, platelet activating factor.

INTRODUCTION

Inflammatory mediators released in the middle ear (ME), induced by viral or bacterial infection, eustachian tube dysfunction, or allergy, seem to play a major role in the pathogenesis of otitis media with effusion (OME). Arachidonic acid (AA) metabolites such as prostaglandins (PGs) and leukotrienes (LTs) have been identified in human middle ear effusion (MEE), as well as in experimentally induced MEEs of animals. They have been shown to play an important role in the pathogenesis of OME.[1-3] Infection, eustachian tube dysfunction, and allergy stimulate release of inflammatory mediators and enzymes such as AA metabolites, histamine, kinin, lyosomal enzyme, hydrolytic enzyme, and protease, which increase vascular permeability and secretory activity, resulting in development of OME.[4,5] The chemotactic activity of these inflammatory mediators displays a self-amplifying effect. Recent studies have also implicated platelet activating factor (PAF) as a potent inflammatory mediator. A biologically active phospholipid, PAF (1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline) is now known to have wide-ranging effects on a number of tissues and inflammatory cells.[6,7] Since the early 1970s, many studies have demonstrated that PAF may play an important role in acute inflammation and allergy. It is released from human neutrophils, platelets, eosinophils, macrophages, mast cells, and vascular endothelial cells.[6,7] Its activities include induction of microvascular leakage, chemotaxis, bronchoconstriction, increased bronchial hyperresponsiveness, increased airway mucus secretion, and induction of hypotension, shock, and death.[6,7] It has been identified in human MEE and has been suggested to participate in developing OME and maintaining MEE by stimulating vascular permeability and chemotaxis.[4] However, the exact role of PAF as an inflammatory mediator in the pathogenesis of OME is not known. The PAF antagonists WEB 2086 and WEB 2170 have been studied extensively and are well proven for their inhibitory effects against various PAF actions[8] (also unpublished abstracts regarding WEB 2170 from Boehringer Ingelheim KG, D-6507 Ingelheim/Rhein, Germany). However, their inhibitory action against PAF-induced OME is not known. The purpose of this study was 1) to test the hypothesis that PAF can induce OME, 2) to establish the dose of PAF necessary to induce OME and the time course of PAF-induced OME, and 3) to test whether PAF antagonists can prevent the development of PAF-induced OME.

From the Division of Otolaryngology–Head and Neck Surgery (Rhee, Jung, Miller) and the Department of Pathology (Weeks), Loma Linda University School of Medicine and Jerry L. Pettis Memorial Veterans Hospital, Loma Linda, California. This study was partially supported by grants from the Department of Surgery, Loma Linda University School of Medicine, and the Deafness Research Foundation. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Loma Linda University School of Medicine.

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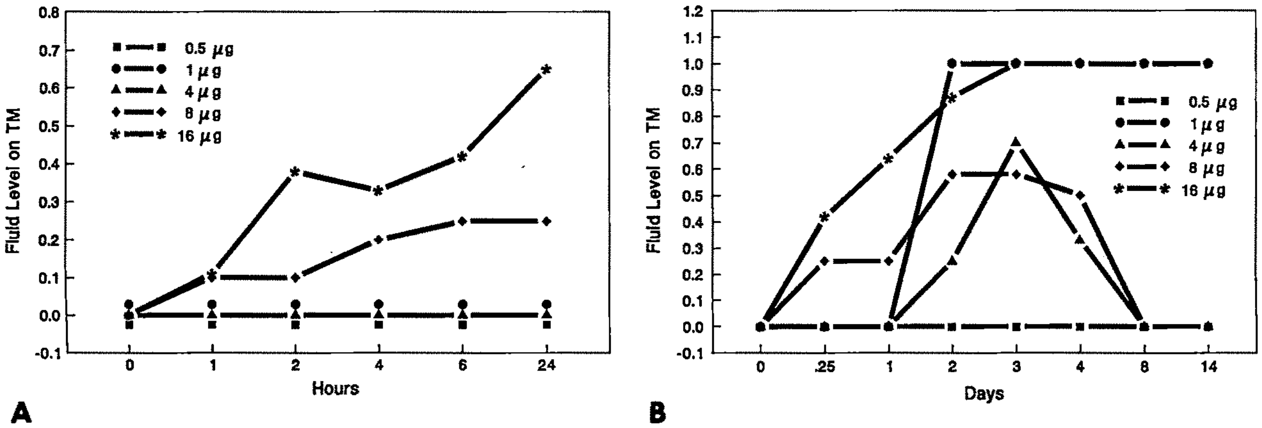

Fig 1. Presence of middle ear effusion (MEE) in experiment 2, measured by fluid level visualized through tympanic membrane (TM) otomicroscopy. A) During initial 24 hours after injection of platelet activating factor (PAF). Groups injected with 8 or 16 μg of PAF developed MEE that increased gradually, while groups injected with 0.5, 1, or 4 μg did not develop MEE within 24 hours. B) For period of 14 days after PAF injection. Groups injected with 1 or 16 μg of PAF developed MEE to top of TM that continued to be present for 14 days. Groups injected with 4 or 8 μg developed partially full MEE that resolved on eighth day. Group injected with 0.5 μg did not develop fluid.

MATERIALS AND METHODS

INDUCTION OF OTITIS MEDIA

All experiments were performed under anesthesia with ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (2 mg/kg).

Experiment 1. Normal saline (NS; 0.5 mL) was injected into the bilateral superior bullae of 5 healthy chinchillas, each weighing approximately 500 g, as a control group. The test group consisted of 5 healthy chinchillas injected with 16 μg of PAF (Sigma, St Louis, Mo) in 0.5 mL of NS into the right superior bullae. The left bullae of this group served as controls, injected with 0.5 mL of NS. At the time of each injection, an air-venting needle was inserted into the superior bullae to prevent excessive pressure buildup. The ears were examined by otomicroscopy daily for 2 days. After 2 days, samples of MEE were aspirated from the bullae, and after sacrifice, its temporal bones were harvested for histopathologic examination.

Experiment 2. Ten healthy chinchillas were randomly assigned to 5 groups, each group receiving intrabullar injections of 0.5, 1, 4, 8, or 16 μg of PAF. By the same technique and manner described in the previous experiment, each dose of PAF was injected bilaterally into 2 chinchillas in each group. Ears were examined by otomicroscopy and tympanometry at 1, 2, 4, and 6 hours, and daily thereafter up to 14 days or until fluid was resolved. From each group, 1 animal was chosen on the 4th day. It had MEE aspirated bilaterally, and after sacrifice, its temporal bones were examined histopathologically. The procedure was repeated with the remaining animals in each group when MEE resolved or by the 14th day.

Experiment 3. Chinchillas were pretreated with intraperitoneal injection of PAF antagonist WEB 2170 (Boehringer Ingelheim, Ridgefield, Conn), 10 mg/kg, 2 hours prior to the intrabullar injection of 1 μg of PAF. The PAF antagonist (WEB 2170) was injected every 12 hours for 48 hours, and the ears were followed by otomicroscopy and tympanometry daily for 2 days. At the end of 2 days, the animals were sacrificed and temporal bones were obtained for histopathologic examination.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY

All samples of aspirated MEE were analyzed for AA metabolites and PAF by high-performance liquid chromatography (HPLC). One hundred microliters of MEE was added to 0.9 mL of ethanol, and the samples were vortexed and centrifuged for 15 minutes at 3,000 rpm. The supernatant was recovered and concentrated to 0.5 mL under nitrogen gas. The ethanolic sample was then dissolved in 9.5 mL of HPLC-grade water and eluted through a C-18 column, Bond Elut/Vac Elut system (Analytical International, Harbor City, Calif). The AA metabolites retained in the cartridge were eluted with 10 mL of methanol. The methanolic eluent was dried and reconstituted with 1 mL of 50% ethanol. The sample (50 μL) was injected into the reverse-phase HPLC. The eluting solvent for lipoxygenase products and PAF was 85% methanol and 15% water (containing 0.2% acetic acid). The flow rate was 1.5 mL/min. The wavelength was set at 280 nm for LTs, 235 nm for hydroxyeicosatetraenoic acids (HETEs), and 205 nm for PAF. The LTs and HETEs were run for 14 minutes, and PAF for 6 minutes. The eluting solvent for cyclooxygenase products was 40% acetonitrile, 60% water (containing 0.2% of acetic acid). The cyclooxygenase products were run for 14 minutes at a flow rate of 2 mL/min. The wavelength was set at

HISTOPATHOLOGIC FINDINGS OF MIDDLE EAR MUCOSA AND BONE

Experimental Group	*Edema*	*Hyperemia*	*Leukocytes*		*Focal Hemorrhage*	*Osteoneogenesis*	*Bone Resorption With Osteoclasts*
			PMN	*MO*			
Exp 1							
5 μg PAF	40 (4/10)	40 (4/10)	10 (1/10)	0 (0/10)	30 (3/10)	0 (0/10)	0 (0/10)
NS	20 (2/10)	40 (4/10)	0 (0/10)	0 (0/10)	40 (4/10)	0 (0/10)	0 (0/1)
Exp 2							
0.5 μg PAF	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
1 μg PAF	100 (4/4)	50 (2/4)	100 (4/4)	100 (4/4)	100 (4/4)	50 (2/4)	50 (2/4)
4 μg PAF	100 (3/3)	0 (0/3)	66 (2/3)	66 (2/3)	0 (0/3)	0 (0/3)	0 (0/3)
8 μg PAF	25 (1/4)	25 (1/4)	50 (2/4)	50 (2/4)	50 (2/4)	0 (0/4)	0 (0/4)
16 μg PAF	100 (3/3)	100 (3/3)	100 (3/3)	100 (3/3)	0 (0/3)	67 (2/3)	67 (2/3)

Data show percentages of ears studied in each group. In parentheses are numbers of ears affected per number of ears studied.

Exp — experiment, PAF — platelet activating factor, NS — normal saline, PMN — polymorphonuclear, MO — mononuclear.

195 nm. The column was a Rainin reverse-phase Dynamax ODS column (4.6 mm x 25 cm) with an ODS guard column. The HPLC system was a Shimadzu binary gradient LC-6A system with a C-R6A data processor and an SPD-6A variable-wavelength ultraviolet detector.

TEMPORAL BONE HISTOPATHOLOGY

After the MEEs were collected, the animals were perfused with buffered 10% formalin solution through a catheter placed in the aorta. The temporal bones were removed, fixed in 10% formalin solution, decalcified in 5% trichloroacetic acid, dehydrated in a graded series of alcohols, embedded in paraffin, and sectioned to a thickness of 15 μm. Every 10th section was stained with hematoxylin and eosin for microscopic examination. To examine the same area from each temporal bone, a small section, approximately 1 cm in diameter, from the anterior-inferior aspect of the temporal bone was removed from each temporal bone and processed by the same method. The presence of edema, hyperemia, leukocyte infiltration, focal hemorrhage, osteoneogenesis, or bone resorption was recorded.

RESULTS

PRESENCE OF MIDDLE EAR EFFUSION

Experiment 1. In the control group, MEE was present in 1 of 10 NS-injected ears (10%) at 24 and 48 hours. In the test group, MEE was present in each of the 5 PAF-injected ears (100%) and 1 of 5 control ears (20%) at 24 hours and 48 hours.

Experiment 2. In the 0.5-μg group, MEE did not develop in up to 96 hours (Fig 1). In the 1-μg group, MEE developed to the top of the tympanic membrane (TM) in 48 hours and lasted fully through 14 days. In the 4-μg group, MEE developed, partially filling the bullae in 48 hours, and lasted up to 8 days. In the 8-μg group, MEE developed within 1 hour, partially filling the bullae and lasting up to 8 days. In the 16-μg group, MEE developed within 1 hour, filling to the top of the TM in 48 hours, and lasted through 14 days. In 1 animal, both TMs bulged and eventually ruptured on the 5th and 11th days, respectively.

Experiment 3. No MEE developed up to 48 hours.

TYMPANOGRAPHY

Experiment 2. All the tympanograms remained as type A for 6 hours and started to shift to types C and B in 24 hours. All the tympanograms correlated well with the presence or absence of MEE, shifting from type A to type C and to type B as fluid accumulated.

Experiment 3. Tympanograms remained as type A or C.

TEMPORAL BONE HISTOPATHOLOGY

A degree of ME mucosal inflammation was present in all experimental groups except the 0.5-μg group, which did not show any inflammatory change (see Table and Fig 2). The inflammatory changes were most severe in the 16-μg group, followed by the 1-μg group after 14 days. Both of these groups demonstrated osteoneogenesis, and the 16-μg group showed bone resorption with osteoclasts. The histopathology of the 16- and 1-μg groups after 4 days showed severe inflammation without osteoneogenesis. The groups injected with 4 and 8 μg showed a moderate degree of inflammation after 4 and 8 days. Mild inflammation was demonstrated in the group injected with 16 μg of PAF or NS (experiment 1) after 48 hours.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY

A wide spectrum of cyclooxygenase and lipoxygenase products was detected. The concentration of PAF in the samples of MEE was higher than the amount of PAF injected. The results of HPLC assay from the group injected with 16 μg of PAF in experiment 2 are shown in Fig 3.

DISCUSSION

This study demonstrates that injection of PAF into

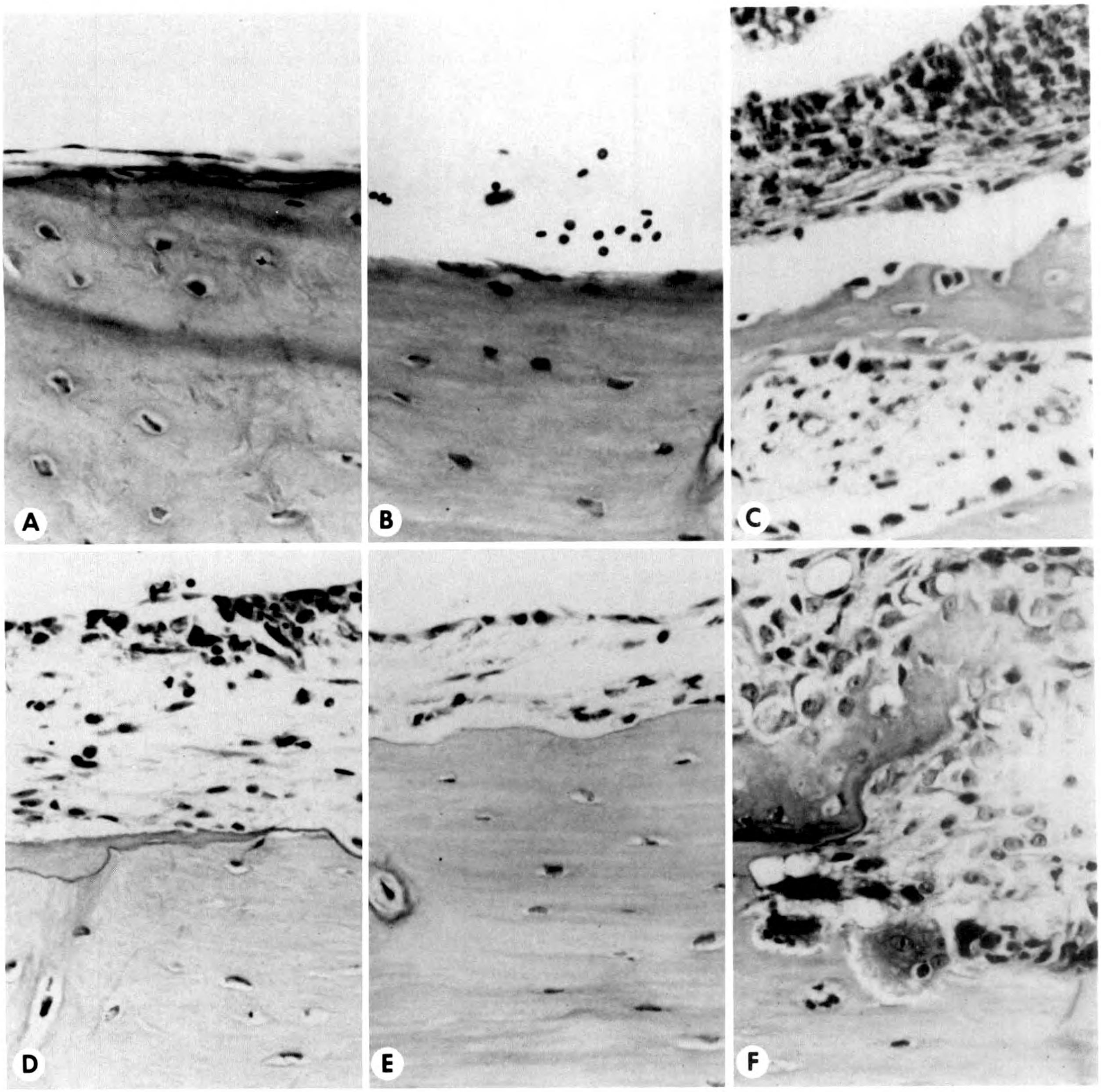

Fig 2. Photomicrographs of chinchilla temporal bones (original x100). A) Right ear (normal saline) from experiment 1 shows very little inflammation. B) Left ear (platelet activating factor [PAF]) from experiment 1 shows inflammatory cell infiltration into middle ear effusion. C) Group with 1 μg of PAF from experiment 2 shows severe inflammation and osteoneogenesis. D) Group with 4 μg from experiment 2 shows moderate inflammation. E) Group with 8 μg from experiment 2 shows moderate inflammation. F) Group with 16 μg from experiment 2 shows severe inflammation with osteoneogenesis and bone resorption.

the ME of chinchillas without obstructed eustachian tubes can cause inflammation and effusion.

In experiment 1, NS induced MEE in 10% to 20% of the NS-injected ears. However, all of the PAF-injected ears developed MEE in 24 hours that continued through 48 hours. This indicates that PAF in the middle ear cavity can induce OME.

In experiment 2, production of MEE was dose-dependent for the first 24 hours. The minimum dose of PAF needed to produce OME was 1 μg. The initial PAF-induced MEE is probably due to increased vascular permeability of the ME mucosa. Further production of MEE and the progression of OME after 24 to 48 hours appeared to be caused by the interaction of various inflammatory mediators produced by the initial chemotactic action of PAF. Leukotrienes and PGs are known to be released from inflammatory cells stimulated by PAF.[9-13] Moreover, PAF has been shown to be released from inflammatory cells and vascular endothelial cells stimulated by various agents, including LTs.[6] At the same time the chemotactic action of PAF may induce infiltration of inflammatory cells such as polymorphonuclear leukocytes,

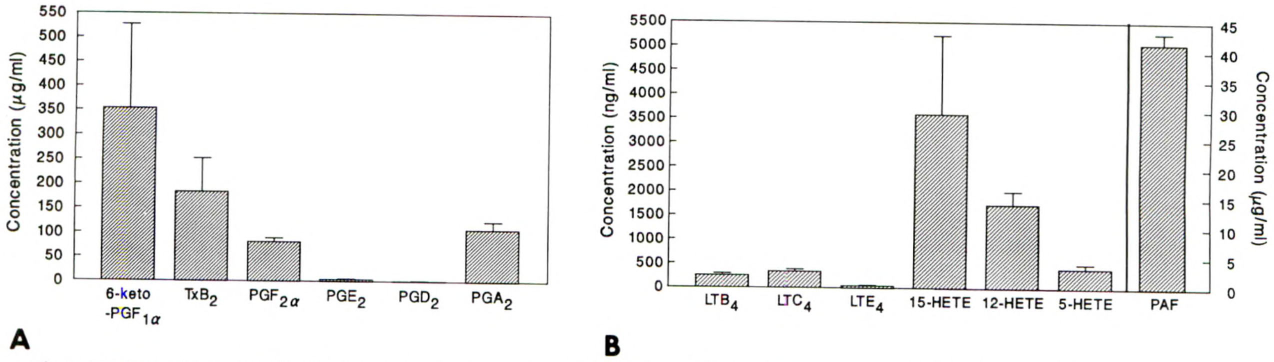

Fig 3. High-performance liquid chromatography assay of middle ear effusions of groups injected with 16 µg of platelet activating factor (PAF). A) Cyclooxygenase products. Dominant products are 6-keto-PGF1α and TxB2. PG — prostaglandin, Tx — thromboxane. B) Lipoxygenase products and PAF. Dominant products are 15-HETE, and LTC4 among the HETEs and LTs, respectively. LT — leukotriene, HETE — hydroxyeicosatetraenoic acid.

monocytes, eosinophils, and basophils into the ME mucosa and into the MEE in the ME space. Eventually, PAF appears to have induced the release of AA metabolites and additional PAF from these inflammatory cells. The presence of these inflammatory cells was well demonstrated in the histopathology of the temporal bones.

In HPLC of AA metabolites and PAF, a wide spectrum of cyclooxygenase and lipoxygenase products and PAF were assayed. Among the cyclooxygenase products, 6-keto-PGF1α and thromboxane B2 were the predominant products. Among the lipoxygenase products, 15-HETE, 12-HETE, and LTC4 were the predominant products among the HETEs and LTs, respectively. The concentration of PAF in the samples of MEE was higher than the injected doses of PAF. The presence of all of the AA metabolites and the high concentration of PAF in the MEE can be explained by the abundant inflammatory cells in the ME mucosa and ME space induced by the chemotactic action of PAF. Interactions of newly released LTs, PGs, and PAF may maintain and regulate the intensity of the inflammatory reaction of the ME. It is not clear why the injection of 1 µg of PAF induced more intense inflammation than did 4 or 8 µg. It may be analogous to an experiment that demonstrated that 1 µg of PAF injected into the rat pleural cavity induced a larger volume of pleural effusion than did higher PAF doses.[14]

In the histopathology of the temporal bones, the ME mucosa from each experiment group showed a mild to severe degree of inflammation. Edema and hyperemia can result from increased vascular permeability and vasodilation due to PAF, PGs, and LTs. Leukocyte infiltration was most pronounced in the 16- and 1-µg groups. Since highly chemotactic PAF and LTs were detected in MEE samples from these groups, it was not surprising to see the dense leukocyte infiltration. Focal hemorrhage was a common feature in the temporal bones from experiment 2; PAF, PGs, and LTs may mediate focal hemorrhage by increasing vascular permeability and vasodilation. Osteoneogenesis, a common histopathologic feature seen in animal models with OM,[3,15] occurred in the 16- and 1-µg groups after 14 days. The significance of this observation is not clear. The most striking feature is bone resorption with osteoclast formation in the 16-µg group after 14 days. Grossly, the temporal bones felt very soft when harvested. It is not clear whether PAF itself or other inflammatory mediators such as PGE2 initiated bone resorptive activity; PGE2, detected in the samples of MEE in this experiment, is well known to induce bone resorption.[16,17]

Investigators have described WEB 2086 as a specific and very potent receptor antagonist of PAF.[8] Chemically related to WEB 2086, WEB 2170 has been tested extensively and had been shown to inhibit various actions of PAF. In this study, WEB 2170 effectively prevented development of PAF-induced OME.

CONCLUSION

This study demonstrated that PAF can induce OME with a minimum dose of 1 µg. Once OME is induced by PAF, it can last over 14 days, as PAF stimulates the release of AA metabolites and additional PAF. The PAF antagonists, such as WEB 2170, effectively prevented development of PAF-induced OME. The findings of this study suggest that PAF plays an important role in the pathogenesis of OME.

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FOURTH RESEARCH WORKSHOP ON THE BIOLOGY, PREVENTION, AND TREATMENT OF HEAD AND NECK CANCER

The Fourth Research Workshop on the Biology, Prevention, and Treatment of Head and Neck Cancer, sponsored by the American Society for Head and Neck Surgery and the Society of Head and Neck Surgeons, will be held in Arlington, Virginia, on September 8-11, 1994. For further information, contact We Plan Meetings, Inc, Ruth C. Enquist, President, 1503 15th St NE, Rochester, MN 55906; telephone (507) 285-1523; fax (507) 281-8328.

Ann Otol Rhinol Laryngol 102:1993

CLASSIFICATION OF CONGENITAL MIDDLE EAR ANOMALIES

REPORT ON 144 EARS

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The surgical findings in 144 successive ears operated on for congenital conductive hearing loss were analyzed, and the results were evaluated in terms of hearing gain. All the patients underwent middle ear surgery at the University Hospital Nijmegen between 1964 and 1990. A classification system was developed to analyze the findings. Class 1 comprises ears with congenital isolated stapes ankylosis. Class 2 comprises ears with congenital stapes ankylosis in combination with a congenital anomaly of the ossicular chain. Class 3 comprises ears with congenital anomalies of the ossicular chain and at least a mobile stapes footplate. Class 4 comprises ears with aplasia or severe dysplasia of the oval window or round window.

KEY WORDS — aplasia, conductive deafness, congenital deafness, congenital malformations, genetic deafness, middle ear, neo-oval window, oval window, round window, stapedial artery, stapes ankylosis, stapes surgery.

INTRODUCTION

There are many different types of congenital ear anomalies, which may be broadly divided into major ear anomalies and minor ear anomalies.[1,2] Minor congenital anomalies are restricted to the middle ear, while major congenital anomalies are malformations of the middle ear and external meatus and sometimes the auricle.

In one quarter of cases of minor ear anomalies, the congenital hearing loss forms part of a syndrome. The great diversity of anomalies of the middle ear that are described in the literature makes it difficult to compare and analyze them without prior classification. Various classification systems have been proposed or suggested in the literature, but in our opinion they leave much to be desired. Therefore, we developed a new classification system and employed it in the presentation of the findings and results of a series of ears operated on up to 1986.[3-5] The application of this classification system to series published by other authors allows reliable comparison of results. The system, presented in Table 1, is based on the different surgical reconstructive techniques available for the anomalies encountered in patients. For example, it makes a difference whether or not reconstructive stapes surgery was performed, because the vestibulum may have been opened. In addition, stapes replacement surgery is more difficult if the incudomalleal complex is malformed. Consequently, a separate group of anomalies is distinguished in which the incudomalleal complex forms the only cause of hearing impairment. Another, separate group comprises ears with aplasia of the oval window or round window, because creating a neo-oval window involves more risk to the inner ear, and it also requires a different surgical procedure.

The objective of this classification is to provide more insight into the frequency with which these isolated or associated congenital middle ear anomalies occur with other anomalies and subsequently to analyze the various surgical techniques. The results of such analyses will provide guidelines for surgical indications, the technique required, or the need to develop new techniques.

In earlier publications we presented the surgical findings and results of the ears from this series, which were operated on in the period up to 1986 because of congenital stapes ankylosis or a congenital anoma-

TABLE 1. CLASSIFICATION OF CONGENITAL ANOMALIES OF MIDDLE EAR

Class	*Main Anomaly*	*Subclassification*	*No. of Ears*
1	Congenital stapes ankylosis		44
2	Stapes ankylosis associated with another congenital ossicular chain anomaly		55
3	Congenital anomaly of ossicular chain but mobile stapes footplate	Discontinuity in ossicular chain	11
		Epitympanic fixation	20
4	Congenital aplasia or severe dysplasia of oval window or round window	Aplasia	10
		Dysplasia	
		Crossing facial nerve	3
		Persistent stapedial artery	1

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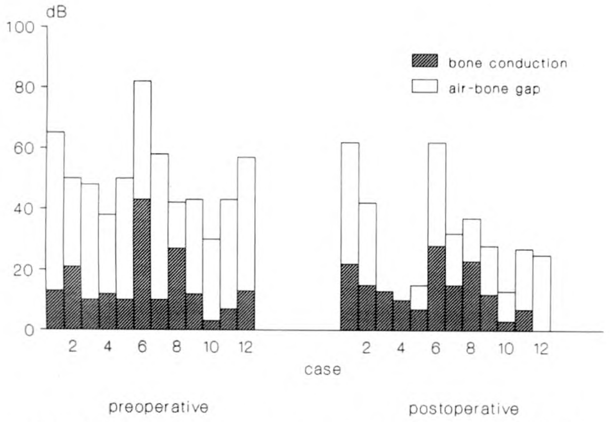

Fig 1. Preoperative and 1-year-postoperative individual hearing levels for bone and air conduction in 12 class 1 ears operated on between 1986 and 1990.

ly of the ossicular chain and a mobile stapes footplate.[3-5] An analysis of the impact of a syndromal diagnosis has already been published.[6] Between 1986 and 1990, 40 ears operated on in 31 patients were added to the series.

PATIENTS AND METHODS

From 1964 to 1990, 144 consecutive ears from 117 patients were operated on because of congenital middle ear anomalies.

This study comprises a retrospective analysis of surgical findings in the period up to 1986 and a prospective part in which the data were incorporated directly into the ongoing study. All patients had a typical history of conductive hearing loss since early childhood. The following inclusion and exclusion criteria were usually employed.

1. The patient was at least 10 years old at the time of the operation.
2. In children, intermittent periods of otitis media with effusion were excluded by a sufficiently long follow-up.
3. The results of pure tone audiometry, speech audiometry, and contralateral stapes reflexes were available.
4. Preoperative computed tomograms were available of the petrous bones; this is especially important in patients with mixed hearing loss.

Over the past 10 years, we have been paying extra attention to syndromal diagnoses to evaluate whether such a diagnosis has any diagnostic or therapeutic impact on ear surgery.

The preoperative and postoperative hearing levels were calculated separately, and the mean levels are presented for the frequencies 0.5, 1, and 2 kHz. Pure tone audiometry was performed routinely 0.5, 1, and 2 years after surgery in all patients and in most cases also once a year for many years thereafter. The hearing levels measured 1 year after surgery were used for statistical analysis of the hearing gain. The air conduction and bone conduction levels were used for the presentation of the results of individual cases, and the air-bone gap levels for the mean hearing gain results. Hearing gain calculations were based on individual values. Preoperative bone conduction levels were not changed in the calculations of cases in which the postoperative bone conduction levels improved. Speech audiometry was performed preoperatively in all patients and, in most cases, also postoperatively.

RESULTS

In this study, the data from 144 ears operated on were classified into 4 groups. The number of ears per group is shown in Table 1.

Preoperative hearing levels and the levels measured at 1-year follow-up are presented in Figs 1-4. The individual results are shown separately for the 4 groups. The Figures present the results of the ears admitted to the study since 1986. The individual

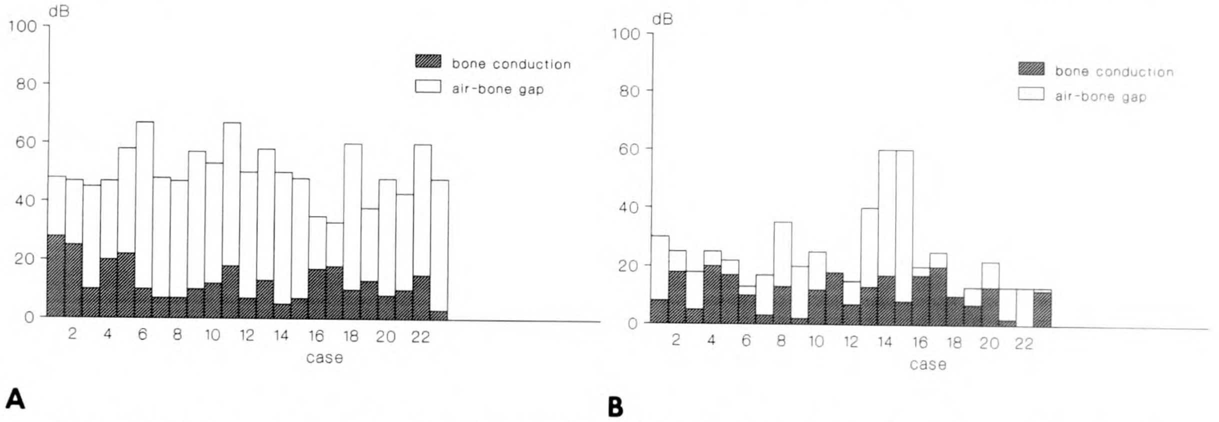

Fig 2. Individual hearing levels for air and bone conduction in 23 class 2 ears operated on between 1986 and 1990. A) Preoperative. B) One year postoperative.

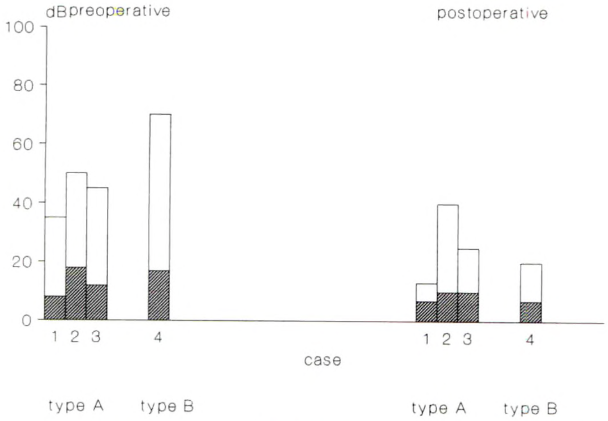

Fig 3. Preoperative and 1-year-postoperative individual hearing levels for bone and air conduction in 4 class 3 ears operated on between 1986 and 1990.

findings and results of the ears in classes 1, 2, and 3 operated on prior to 1986 have been reported in earlier publications.[3-5] The preoperative hearing levels of the ears in class 4 are presented for the whole period from 1964 to 1990 (Fig 4), because the results of these ears with aplasia of the oval window, operated on prior to 1986, have not been published before. In 4 of the 14 ears in class 4 (Fig 4; cases 1, 6, 12, and 14), an attempt was made to improve the patients' hearing; these operations were conducted in 1965, 1975, 1983, and 1989, respectively. The postoperative results at 1-year follow-up are also presented. The number of ears per class and the percentages of air-bone gap closure are shown in Table 2.

Stapes surgery was performed in 88 of the 99 ears in classes 1 and 2. A different type of stapes surgery was necessary to mobilize the stapes in 4 other ears: 2 with a bony stapedial ligament[7] and 2 in patients with Treacher Collins syndrome in whom the stapedial head was fixed to the bony facial canal. In 7 ears, no reconstructive surgery was performed because of a stapes gusher[8] or an aberrant facial nerve.[9] In 26 of the 88 stapedectomies, stapes surgery was combined with additional surgical treatment for the associated congenital anomaly of the ossicular chain. All 26 ears were classified as class 2.

In some of the other class 2 ears operated on, stapes surgery was complicated by anomalies of form during insertion of the replacement stapes prosthesis, or even by the absence of the incus.

If the long process of the incus is too short, it may be necessary to place a malleus footplate prosthesis. A recently developed technique that is only performed in exceptional cases is myringochordavestibulopexy. In this technique the prosthesis is fixed to the chorda tympani by interposing cartilage between the tympanic membrane and the chorda.[10]

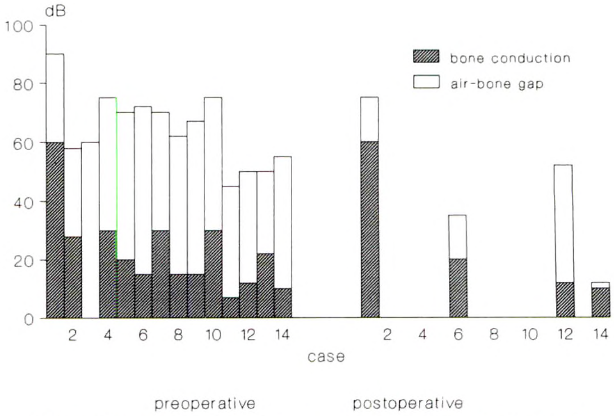

Fig 4. Preoperative and 1-year-postoperative individual hearing levels for bone and air conduction in 14 class 4 ears, including 4 ears with neo–oval window, operated on between 1964 and 1990.

In class 4, 4 neo–oval windows were created. In 3 cases Teflon or Teflon wire was interposed between the incus and the neo–oval window, which had been opened in the promontory[11] (Fig 4; cases 1, 6, and 12), and in 1 case myringochordavestibulopexy was performed[10] (Fig 4; case 14).

When the results of stapes surgery in the 12 ears in class 1 were analyzed, it was striking that there was no improvement in 1 ear (case 1) and that the postoperative threshold remained poorer than 35 dB in 4 ears and poorer than 30 dB in 5 ears. The postoperative bone conduction thresholds in 3 ears (Fig 1; cases 1, 3, and 7) were less favorable after the operation, ranging from a few decibels Fletcher index to 10 dB.

Comparing the results of stapes surgery in class 2 in Fig 2A and B showed that there was no hearing gain in 2 ears (cases 14 and 15). A considerable hearing gain was achieved in all 21 of the other ears. In 2 ears (cases 22 and 23) there was only a very slight improvement in the perceptive component of 10 dB or less postoperatively.

Only 4 ears were added to class 3 after 1986, and the hearing of all of them improved. In 1 ear (Fig 3; case 2) the hearing gain remained limited, with a Fletcher index of more than 40 dB. There was no increase in the perceptive threshold postoperatively.

In class 4 we confirmed the current view in some of the reports in the literature that creating a neo–oval window is associated with a greater risk of paralysis of the inner ear. Fairly recently, a shift has occurred in these limitations, and the creation of a neo–oval window can once again be considered as a form of ossicular chain reconstruction.

In 1965 an attempt was made to create a neo–oval window in 1 case (case 1), without any notable success, and no other attempts were made until 1975

TABLE 2. AIR-BONE GAP 12 MONTHS AFTER RECONSTRUCTIVE SURGERY IN 127 PATIENTS WITH CONGENITAL MIDDLE EAR ANOMALIES

Class	No. of Ears	No. With Reconstructive Surgery	Air-Bone Gap <10 dB	Air-Bone Gap 10-20 dB	Air-Bone Gap >20 dB
1	44	41	20 (49%)	9 (22%)	12 (29%)
2	55	51	20 (39%)	17 (33%)	14 (27%)
3	31	31	9 (29%)	14 (45%)	8 (26%)
4	14	4	1 (25%)	2 (50%)	1 (25%)

(case 6). The hearing threshold improved from 70 dB to 35 dB, and the perceptive threshold remained about the same. In case 14 (1989) of this series, the good result is evident.

DISCUSSION

This series of 144 operated middle ear anomalies showed great variation in the nature and severity of congenital anomalies. Therefore, diverse surgical procedures were applied to improve the patients' hearing. To achieve a more easily surveyable presentation of the findings and results, we sought a practical classification system. In the literature, various authors have proposed classification systems based on their own operated cases (Table 3[2,12-14]).

Within this great variation, we distinguished 4 main classes. First, this classification preselects anomalies on the basis of the condition and mobility of the stapes and the stapes footplate. Classes 3 and 4 are subdivided further.

The clinical findings and results of ear surgery have been published separately for 3 of the 4 main classes.[3-5] The most important reason for this is to achieve a surveyable presentation and description of the great diversity of congenital middle ear anomalies and at the same time to present the results in relation to ear surgery for each separate ear and for each class. This made it much easier to compare the results of congenital middle ear anomalies and also the various types of reconstructive surgery of the ossicular chain.

Class 1 comprises ears with isolated congenital stapes ankylosis, and class 2 the operated ears with congenital stapes ankylosis and an associated anomaly of the rest of the ossicular chain.

These classes of anomalies have the same embryologic basis. The earlier-induced thinning of the bony division between the otic capsule and the stapes develops into the stapes footplate. Subsequently, there is further deossification of the annular ring around the stapes, and the stapes becomes mobile. Disruption of this process during the development of the ear leads to an anomaly in which the footplate is found to be thickened and fixed to the annular ring.[15]

Stapes ankylosis has also been accepted as a separate entity by authors. Several reports have appeared in which the results of operative treatment for stapes ankylosis were published separately.[13,14,16,17] However, none of the previous authors distinguished between patients with isolated stapes ankylosis and patients with stapes ankylosis with an associated congenital ossicular chain anomaly.

The surgery of choice for isolated stapes ankylosis is regular stapedectomy, in which the prosthesis is attached to the mobile incus. It was striking that the mean postoperative hearing gain in the patients with a deformed stapes suprastructure and isolated stapes ankylosis was 11 dB lower than that in the patients with a normal suprastructure.[3] We could not find any explanation for this.

Class 1 comprises 2 additional special forms of isolated stapes fixation other than at the level of the stapes footplate itself: fixation of the stapedial head to the bony facial canal and fixation of the stapes owing to a bony stapedial ligament. In both these situations, there were no other anomalies of the ossicular chain. The anomaly in which the stapedial head was fixed to the bony facial canal was only encountered in patients with Treacher Collins syndrome. The application of laser surgery to such cases in the near future may achieve greater hearing gain.

Class 2 comprises operated ears with congenital ankylosis and an associated anomaly of the incus or malleus. It is sometimes difficult to recognize this anomaly during surgery. For instance, fixation of the short process of the incus in the fossa incudis can easily be overlooked. Such ears are then wrongly classified as class 1 ears, because the associated anomaly was missed by the surgeon, and will not number among the successfully operated ears in class 1.

Surgical treatment of this associated anomaly is essential to achieve the required level of hearing gain. Fixing the prosthesis during malleovestibulopexy will lead to problems in a number of cases, particularly when the incus and malleus handle are completely absent. Moreover, fixation of the incus and/or malleus will have to be corrected first; this procedure can take place in the same session. Various modifications have been made to the malleovestibulopexy technique over the past 10 years, including the availability of a 0.4-mm Teflon-platinum piston and an alternative method of fixing the malleus.

Class 3 is characterized by a mobile stapes footplate.

TABLE 3. EARLIER CLASSIFICATIONS PROPOSED FOR CONGENITAL MIDDLE EAR ANOMALIES

Henner and Buckingham[12]	Class 1	Minor ear anomalies
		Ossified stapedial tendon
		Ossified ligament
		Synostosis of incus-malleus joint
		Discontinuity of ossicular chain
		Agenesis of long process of incus
		Agenesis of stapes crura
		Agenesis of annular ligament of stapes footplate (stapes ankylosis)
		Agenesis of stapes and footplate (aplasia of oval window)
	Classes 2 and 3	Major ear anomalies
House[13]		Congenital stapes ankylosis
		Congenital ossicular chain discontinuity
		Congenital fixed malleus syndrome
		Congenital fixed incus syndrome
		Congenital ossified stapedial tendon
Arslan and Giacomelli[14]		Normal
		Absence of long process of incus
		Aplasia of oval window and footplate
		Aplasia of oval window, footplate, and stapes
		Aplasia of oval window, footplate, and stapes and fixation of incus-malleus joint
Ombrédanne[2]		Congenital ossicular chain discontinuity
		Congenital ossicular chain ankylosis
		Congenital aplasia of oval window and stapes

The congenital anomaly in these cases lies in a deformed stapes, incus, or malleus suprastructure, which causes discontinuity of the ossicular chain (type 3A). Anomalies involving fixation of the incus or malleus but a mobile stapes are also classified as class 3 (type 3B). These anomalies are considered to be variations of developmental disorders of the first and second branchial arches. Very few published series are available in the literature that describe cases comparable to class 3.[12] From an otologic point of view, the surgical treatment for this anomaly is fundamentally different from that for classes 1 and 2, because the vestibulum is not opened. Reconstruction of the ossicular chain can be achieved in various ways, whether or not in combination with mobilization of another part of the ossicular chain. There is so much variation between the anomalies and the required surgical techniques that we decided not to make any further subclassifications. An anomaly in which fixation of the ossicular chain is caused by a bony plate in the anterior upper quadrant of the tympanic membrane, between the malleus handle and the bony anterior annulus, is included in class 3. This anomaly can also be considered a mild form of congenital aural atresia. It therefore occupies the transitional area between the major and minor congenital ear anomalies.

Class 4 comprises aplasia of the oval window and/or round window. This anomaly forms such a clear clinical entity that it was desirable to create a separate group for its classification. There is no clear embryologic explanation for this anomaly. It has been suggested that during normal development, the bony division between the inner and middle ears gradually becomes thinner, starting at the otic capsule at the level at which the stapes develops in the middle ear.[15] At the same time, the stapes takes on its definitive form. It is probable that the developing stapes induces the thinning of the bony division that later becomes the stapes footplate. In the same way, the otic capsule will influence the development of the stapes. If this complex mechanism is disrupted during the course of development, various congenital anomalies of the oval window and stapes may arise. The most extreme form, total aplasia of the oval window, is often seen in association with aplasia or severe deformity of the stapes, in which the stapedial processes, if present, hardly ever reach the region of the footplate. This observation supports the above description of embryologic development. However, other authors still regard aplasia as a separate entity.[2,18] So far, there is no definite answer to the question of which surgical technique is the best treatment for aplasia of the oval window. The greatest drawback is that opening the vestibulum can lead to considerable damage to the inner ear function. Ombrédanne[2] usually performed fenestration of the semicircular canal. Later an interpositioning procedure was applied, in which the promontory was opened.

In our own series, there were 14 cases of aplasia of the oval window. A neo–oval window was created in 4 of them with Teflon interpositioning, the first in

1965; 2 were successful.[11]

Class 4 also comprises a number of anomalies that are denoted as serious dysplasia of the oval window. In these anomalies, the oval window is completely obscured by overlying facial nerve or a persistent stapedial artery. Successful neo–oval window procedures were described by Sterkers and Sterkers[19,20] in 6 ears with aplasia of the oval window and an aberrant facial nerve. The neo–oval window was not created on the promontorial side, but on the cranial side of the facial nerve.

In about 1 of every 4 ears operated on, the congenital middle ear anomaly is not isolated, but forms part of a syndromal diagnosis.[6] It is also useful for the ear surgeon to be aware of the syndromal diagnosis. For instance, at present it is possible to diagnose the X recessive syndrome with a stapes gusher preoperatively and therefore not perform surgery.[21] In the case of the branchiogenic syndrome, the surgeon will anticipate more complex and more severe anomalies of the middle ear and inner ear. The diagnosis of craniosynostosis implies that the epitympanum may be too small and stapes surgery may have to take the form of malleovestibulopexy.[6]

Our series of 144 consecutive congenital middle ear anomalies is notable for its size and the proposed classification system. We have arranged our data in such a way that for each ear the preoperative and postoperative hearing thresholds for air and bone conduction are presented, partly in earlier publications on the surgical results of the subclasses,[3-5] making it possible to evaluate the results in relation to the actual middle ear anomaly and the surgical technique applied.

A survey of the results of the whole series showed that the most disappointing results occurred at the beginning of the series, in the mid-1960s, in ears with major mixed hearing losses. It appears that in later years the level of the sensorineural element of the hearing loss formed an unobtrusive selection criterion. Another interesting finding is that nearly all the ears had a preoperative and postoperative sensorineural loss of 10 to 20 dB. In the operative evaluation of such patients, the surgeon may feel inclined to consider this component as an artifact of the bone conduction threshold measurement, but this is not the case. The bone conduction thresholds of patients with a minor congenital abnormality are seldom normal, while these are mostly normal in patients with a major congenital ear anomaly. It is important to realize this when predicting the degree of success of an operation. Our results (Snik, Teunissen, and Cremers, unpublished observations) have shown that the hearing gain achieved, even in cases with a unilateral major or minor congenital anomaly, is highly beneficial in terms of tone and speech recognition.

The total results demonstrate unmistakably that ear surgery is a worthwhile treatment for congenital middle ear anomalies, particularly when intervention is limited to classes 1, 2, and 3 and the selection criteria are strictly adhered to. In a few specific cases requiring ossicular chain reconstruction or malleovestibulopexy because of a congenital middle ear anomaly such as aplasia of the oval window and/or round window, it is not yet clear what the best treatment is and, if surgery is indicated, which surgical technique is the most appropriate. Aside from this, the cogency of our results would be enhanced by similar studies from other centers.

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Crespo PV, Lopez-Escamez JA, Canizares FJ, Campos A. (Granada, Spain) X-ray microanalytical determination of P, S and K concentrations in the gelatinous membrane of the utricle.

Ikeda K, Sunose H, Takasaka T. (Sendai, Japan) Effects of free radicals on the intracellular calcium concentration in the isolated outer hair cell of the guinea pig cochlea.

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499: *Iurato I, ed.* Multicentre evaluation of the temporal bones obtained from a patient with suspected Meniere's disease.

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Somnolence	8	6	22	9
Fatigue	4	3	10	2
Dry Mouth	3	2	4	3

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Autonomic Nervous System Altered salivation, increased sweating, altered lacrimation, hypoesthesia, impotence, thirst, flushing.
Body As A Whole Conjunctivitis, blurred vision, earache, eye pain, tinnitus, asthenia, weight gain, back pain, leg cramps, malaise, chest pain, rigors, fever, aggravated allergy, upper respiratory infection, angioneurotic edema.
Cardiovascular System Hypotension, hypertension, palpitations, syncope, tachycardia.
Central and Peripheral Nervous System Hyperkinesia, blepharospasm, paresthesia, dizziness, migraine, tremor, vertigo, dysphonia.
Gastrointestinal System Abdominal distress, nausea, vomiting, flatulence, gastritis, constipation, diarrhea, altered taste, increased appetite, anorexia, dyspepsia, stomatitis, toothache.
Musculoskeletal System Arthralgia, myalgia.
Psychiatric Anxiety, depression, agitation, insomnia, paroniria, amnesia, impaired concentration, confusion, decreased libido, nervousness.
Reproductive System Breast pain, menorrhagia, dysmenorrhea, vaginitis.
Respiratory System Nasal dryness, epistaxis, pharyngitis, dyspnea, nasal congestion, coughing, rhinitis, hemoptysis, sinusitis, sneezing, bronchospasm, bronchitis, laryngitis.
Skin and Appendages Dermatitis, dry hair, dry skin, urticaria, rash, pruritus, photosensitivity reaction, purpura.
Urinary System Urinary discoloration, altered micturition.

In addition, the following spontaneous adverse events have been reported rarely during the marketing of loratadine: peripheral edema; abnormal hepatic function including jaundice, hepatitis, and hepatic necrosis; alopecia; seizures; breast enlargement; erythema multiforme.

OVERDOSAGE
Somnolence, tachycardia, and headache have been reported with overdoses greater than 10 mg (40 to 180 mg). In the event of overdosage, general symptomatic and supportive measures should be instituted promptly and maintained for as long as necessary.

Treatment of overdosage would reasonably consist of emesis (ipecac syrup), except in patients with impaired consciousness, followed by the administration of activated charcoal to absorb any remaining drug. If vomiting is unsuccessful, or contraindicated, gastric lavage should be performed with normal saline. Saline cathartics may also be of value for rapid dilution of bowel contents. Loratadine is not eliminated by hemodialysis. It is not known if loratadine is eliminated by peritoneal dialysis.

Oral LD_{50} values for loratadine were greater than 5000 mg/kg in rats and mice. Doses as high as 10 times the recommended clinical doses showed no effects in rats, mice, and monkeys.

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AUSTRALIAN EXPERIENCE WITH FUNCTIONAL ENDOSCOPIC SINUS SURGERY AND ITS COMPLICATIONS

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There have been reports of severe complications from functional endoscopic sinus surgery; however, it is unclear as to how the rate compares with that of the more traditional forms of intranasal surgery. To ascertain this, I circulated a questionnaire to 100 otolaryngologists in Australia known to have an interest in endoscopic nasal surgery and analyzed the results. The results of this survey show that complications are very infrequent and that the rates for functional endoscopic sinus surgery compare favorably with those of the traditional techniques.

KEY WORDS — complications, functional endoscopic sinus surgery.

Functional endoscopic sinus surgery (FESS), in common with the traditional types of ethmoid surgery, is associated with occasional complications — some severe. However, it is unclear how the complication rates of these two forms of surgery compare, particularly given the fact that most practitioners of FESS are currently crossing over to the new technique and thus will be relatively inexperienced. An idea of the expected complication rate should be known so that a patient can gain informed consent before submitting to this surgery.

It was thought that Australia would be an ideal nation to gain relatively accurate information of complication rates through the circulation of a questionnaire to those otolaryngologists known to have an interest in the field. Australia is a closed island nation with a population of 17.5 million people and with a homogeneous group of 270 well-trained otolaryngologists with access to first-class facilities. The otolaryngology community is in constant contact with one another through social and professional gatherings, and almost all of the major complications reported by respondents to the questionnaire were already known by the author, and most of the lesser ones as well. This fact would therefore tend to substantiate the validity of these results.

The questionnaire (Table 1) was circulated in May 1992 to 100 otolaryngologists, and there were 72 respondents. Basically, what was asked was their experience with the technique, that is, the number of procedures that they had performed; whether they mostly used general or local anesthesia for the surgery; what preliminary self-educative processes they had undertaken before commencing the surgery; and what complications they had experienced.

In the absence of any universally adopted classification of the degree of sinus disease in patients, no attempt was made to request of respondents that they classify the degree of sinus disease operated on, and thus this could not be related to the complications reported. Similarly, no attempt was made to define the extent of surgery performed and how this related to complication rates.

It would appear from the results that over 10,000 FESS operations have been performed in Australia since the mid-1980s. It should be noted that this figure refers to patients and not sides operated on, which would be expected to be twice this number. It is of the author's opinion that any discussion of complication rates should relate to the number of patients operated on and not to procedures (sides) performed, as has often been done in the literature.[1-7] Most patients present to a surgeon with nose rather than nostril symptoms, and the vast majority of operations performed will be bilateral. Patients, in gaining informed consent, are only interested in percentages that relate to them as patients, not a figure halved by quoting sides or procedures.

Virtually all respondents had attended a basic FESS course, either in Australia or abroad, and the majority had performed cadaveric dissections in a hospital or a state mortuary. All respondents who reported complications had attended a basic FESS course, and only 1 had not done further cadaveric dissection in a mortuary.

The majority of respondents favored general anesthesia over local anesthesia. There is a strong bias toward general anesthesia in Australia in surgery, and this may be partly related to the fact that most anesthetists in Australia are British-trained or certainly

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From the Royal Victorian Eye and Ear Hospital, Melbourne, Australia.

Presented at the First International Advanced Course of Functional Endoscopic Sinus Surgery, Vienna, Austria, July 29–August 1, 1992.

TABLE 1. QUESTIONNAIRE

1.	What number of FESS procedures have you performed?	Please circle one or insert number 0-50 50-100 100-300 If more than this, approx. No. ______	
		Please circle the relevant one below	
2.	Do you mostly use GA or LA?	GA	LA
3.	Have you attended a basic FESS course?	Yes	No
4.	Have you performed cadaveric dissections in a mortuary?	Yes	No
5.	Do you feel that FESS has been a significant advance in the treatment of sinusitis?	Yes	No
6.	What approximate percentage of your primary FESS procedures do you revise?	____ %	
7.	Do you think that FESS is complementary or replacement treatment to the traditional surgical treatments of nasal and sinus conditions?	Complementary	Replacement
8.	Have you had any of the following complications?		
	1. Adhesions (comment)		
	2. Damage to lacrimal apparatus (comment)		
	3. Hemorrhage (comment)		
	4. Ocular complications (comment)		
	5. Intracranial complications including CSF leak (comment)		
	Any other comments		
NAME:	(Optional)		

FESS — functional endoscopic sinus surgery, GA — general anesthesia, LA — local anesthesia, CSF — cerebrospinal fluid.

British-influenced, and in Britain there is a similar preference. For example, most stapedectomies and rhinoplasties in Australia are performed under general anesthesia.

All respondents agreed that FESS had been a major contribution to the diagnosis and treatment of sinusitis. Most agreed that FESS was a complementary treatment for sinusitis, rather than one that totally replaces all existing and traditional forms of treatment.

COMPLICATIONS

The majority of respondents had had problems with adhesions. Some claimed that this problem had been diminished with stenting, while others sutured the middle turbinate to the septum, claiming similar improved results. There were 15 reported cases of postoperative hemorrhage, none requiring transfusion, and all settled quickly with local treatment or nasal packing. There were 15 reported cases of lacrimal symptoms following surgery. Most were temporary, with transient epiphora, but 3 went on to require a dacryocystorhinostomy. If we take these 3 as being severe complications, then the overall complication rate (in 10,000 cases) is 0.03%.

Ocular Complications. There were 12 reported cases of ocular complications (Table 2), excluding periorbital discolorations and hematomas. Eight of these involved intraorbital bleeding, and 3 were temporary medial rectus diplopia. There was 1 case of optic nerve trauma. The outcome of the ocular complications is shown in Table 2. Of the 8 intraorbital bleeds, 2 patients had an external ethmoidal ligation, and 1 required a lateral canthotomy. The other 5 were treated expectantly with no sequelae. Of the 3 who required surgical intervention, 2 have persistent medial rectus tethering. All 3 cases of immediate medial rectus diplopia as a sole symptom resolved. The case of optic nerve damage remains permanent. This gave an overall ocular complication rate of 0.12%, and patients with severe ocular sequelae (ie, diplopia to blindness) totaled 0.03%.

Intracranial Complications. The only intracranial complications were cerebrospinal fluid (CSF) leaks, and there were 22 of these. Fourteen were recognized at the time of surgery and were immediately patched, plugged, or packed, with no sequelae. Of the other 8 cases, 5 required subsequent closure, which was achieved mostly by endoscopic or external flap repair. Three cases closed with conservative management, although 1 of these patients developed meningitis that settled on appropriate treatment. The overall leak rate was 0.22%, and if one isolates those leaks that required subsequent surgical treatment, the rate is 0.05%.

DISCUSSION

There is a wide variation in the reported complication rates from FESS. These vary from a complication rate of 29% for the first 150 procedures, as

TABLE 2. OCULAR COMPLICATIONS (TOTAL OF 12) AND THEIR OUTCOME

Intraorbital hemorrhage			8
Anterior ethmoid ligation		2	
Lateral canthotomy		1	
Conservative treatment		5	
Sequelae			
Complete resolution	5		
Medial rectus tethering	2		
Poor vision	1		
Medial rectus diplopia (only)			3
Resolved		3	
Optic nerve damage			1
Permanent		1	

Overall ocular complication rate was 0.12% (in 10,000 cases). Rate of severe ocular sequelae (ie, diplopia to blindness) was 0.03%.

reported by Stankiewicz,[8] to a series of over 4,000 patients from Graz, Austria, where minimal complications have occurred, as reported by Stammberger and Wolf.[9] There are also anecdotal reports of high complication rates from less developed Third World nations in which the procedure has been recently introduced.

Part of this disparity may be explained by the extent of surgery performed. The original Messerklinger technique[10] was essentially minimalist surgery, often with only local excision of an uncinate process or ethmoid bulla to complete the procedure. Anybody who has witnessed Dr Heinz Stammberger, who was trained by Professor Messerklinger, perform this surgery will note that only a few milliliters of blood are lost, and the overall philosophy of the surgery is that it be performed for ventilation of the major sinuses.

However, another surgical school teaches that total exenteration of diseased ethmoid cells is necessary to gain nasal and sinus health. Therefore, this surgery is more extensive and is associated with increased blood loss, thus making visibility more difficult and increasing the likelihood of serious intracranial or orbital penetration. It should be noted that several of the patients reported on by Stankiewicz[8] lost over 1,200 mL of blood intraoperatively.

With regard to CSF leaks, I suspect that the reported intraoperative rate will be greater with endoscopic surgery than with the traditional intranasal technique, because the leaks can be so quickly and readily diagnosed. It is to be noted that of the 22 CSF leaks reported in this series, 14 were diagnosed intraoperatively and dealt with immediately. It is probable that many of these leaks would not have been spotted with the traditional technique and would have healed spontaneously. The majority of the CSF leaks reported with traditional techniques are those that persist after surgery. In our survey the rate of CSF leaks requiring treatment postoperatively compared favorably with that of reported series of traditional intranasal surgery.[1-5,7,11]

CONCLUSION

A national complication rate for FESS is presented that represents those complications that an otolaryngologic surgeon population has experienced as it has educated itself into a new surgical technique. It is expected that this complication rate, though comparing favorably with traditional techniques, will fall even lower as the technique is introduced into resident training programs and current practitioners gain more experience.

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DIFFERENTIAL OSTEOCLAST ACTIVATION IN ENDOCHONDRAL AND INTRAMEMBRANOUS BONE

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DAVIS, CALIFORNIA

Osteoclast activation within the temporal bone leads to hearing loss in a variety of pathologic conditions, including otosclerosis, osteogenesis imperfecta, Paget's disease of bone, and chronic otitis media. Although endochondral (EC) bone is remodeled in these diseases, it is relatively more resistant to remodeling when compared to intramembranous (IM) bone. In this study, localized bone resorption and remodeling were induced on the inner surface of the middle ear by pressurization with air to 10 mm Hg above atmospheric. Osteoclast surface increased in IM bone, but not in EC bone. This study supports the concept that there may be fundamental differences between EC and IM bone and that EC is more resistant to pressure-induced localized resorption than IM bone.

KEY WORDS — endochondral bone, intramembranous bone, osteoclast.

A bone modeling and remodeling process within the middle ear and inner ear leads to hearing and vestibular decrement in a variety of pathologic conditions such as otosclerosis, osteogenesis imperfecta, Paget's disease of bone, and chronic otitis media. These processes appear to affect both the endochondral (EC) bone of the otic capsule and the intramembranous (IM) bone of the mastoid. In some cases, the EC bone appears to be more resistant to erosion than does the IM bone, eg, chronic otitis media. In other cases the remodeling process principally involves EC bone. For example, osteoclast activation within the EC bone of the human otic capsule appears to be the initial event in a localized remodeling process that results in an otospongiotic or otosclerotic lesion.[1] Normally, the EC bone of the otic capsule calcifies in its adult conformation and does not undergo remodeling; osteoblast-osteoclast bone remodeling units have not been seen in normal otic capsular bone. Endochondral bone forms by calcification of primitive cartilage as in the epiphyseal plate of long bone or the otic capsule; IM bone forms by calcification within primitive fibrous tissue as in the calvarium. In otosclerosis, the adult otic capsule remodels in a pathologic manner, causing stapes fixation and possibly inner ear injury. Hence, EC and IM bone may respond differently in certain pathologic conditions within the temporal bone.

The remodeling process can be induced within the IM bone of the middle ear of experimental animals by pressurization with air[2] in which osteoclastic activity can be induced within 48 hours.[3] In this model the entire interior surface of the middle ear is subjected to air pressurization and the IM bone of the ventral surface is evaluated for osteoclast number and surface. Although not the subject of prior studies using this model, EC bone of the medial wall of the middle ear (the cochlear otic capsule) is subjected to the same pressures as the IM bone of the remaining middle ear. In this study we hypothesized that EC bone would be less sensitive to osteoclastic resorption than adjacent IM bone on the basis of qualitative anatomic observations in related studies.

MATERIALS AND METHODS

Ten 90- to 110-day-old male Mongolian gerbils, *Meriones unguiculatus*, were purchased from Tumblebrook Farms (West Brookfield, Mass) and placed in individually numbered cages. Inflow-outflow implant catheters and shams were manufactured from 18-gauge by 3.8-cm stainless steel hypodermic needles (Monoject, St Louis, Mo) and shaped for attachment to the dorsum of the gerbilline skull. Segments of polyethylene tubing (PE-160 Intramedic; Clay Adams, Parsippany, NY) and 3-mm–outside diameter, 30-cm-long stainless steel springs (Small Parts, Inc, Miami, Fla) were used to attach each animal to the central air pressure line.

Each animal underwent catheter and sham tube implantation under continuous anesthesia with 2,2-dichloro-1, 1-difluoro-ethylmethyl ether (Metofane; Pittman-Moore, Mundelein, Ill).

A diamond-tipped, high-speed dental drill was then used to make 3 holes 1.2 mm in diameter in the dorsolateral bulla of each side: 2 holes on the right

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From the Department of Otolaryngology, University of California at Davis, Davis, California. Supported by a grant from the National Institutes of Health (RO1 DC00263-06). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California at Davis.

REPRINTS — Richard A. Chole, MD, PhD, Otology Research Laboratory, 1159 Surge III, University of California at Davis, Davis, CA 95616.

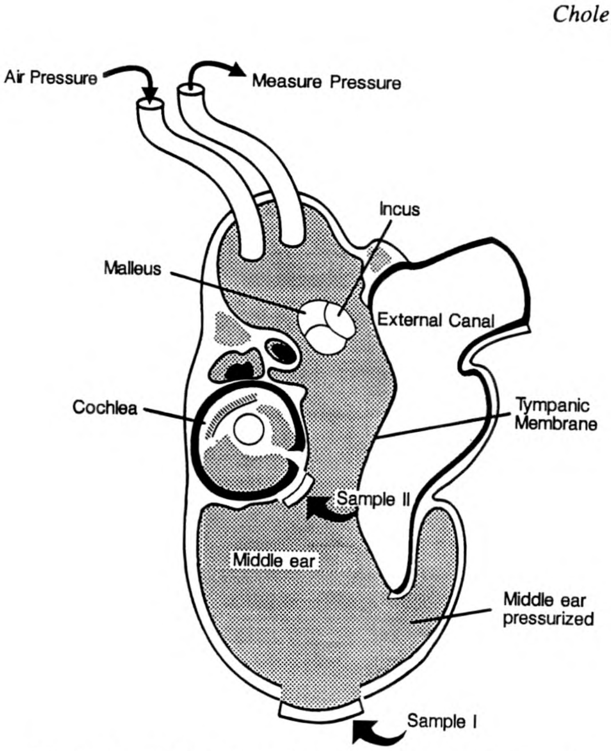

Fig 1. Sample locations shown on diagram of coronal section through gerbil bulla. Sample I — intramembranous bone, sample II — endochondral bone.

(experimental pressure side) and 1 hole on the left (control side). Two shallow holes were also drilled in the dorsomedial calvarium to allow placement of 2 No. 0-80, 3-mm anchoring screws (Small Parts, Inc) to stabilize the headset.

The stainless steel inflow and outflow catheters were then fitted into the 2 holes of the right bulla and extended to a central position over the dorsomedial skull. The sham tubes were likewise fitted into the single holes of the left bulla. Dental acrylic (Grip Cement; R & R Dentsply, Maumee, Ohio) was applied to embed the catheters and screws in order to bond them securely to the skull. The inflow and outflow catheters were then connected to the polyethylene tubing encased in the protective 3-mm spring sheaths and secured to the head-mount with additional cement.

Once the acrylic had hardened and the animals had fully recovered in warmed cages, the gerbils were transferred to modified metabolic cages, where their pressure tubings could be extended and attached to swivel units mounted over the tops of the cages. The animals were thus independently attached to the central air pressure line and allowed a full range of motion. A constant central air pressure was supplied by a diaphragm-driven air pump with pressure regulated to 10 mm Hg by a water column. Hence, a constant 10 mm Hg of air pressure was supplied from the central air line to each animal's right bulla via the appropriate middle ear inflow tube. The outflow tubes were blocked by removable plugs to allow measurement of actual pressures and to ensure patency. Adequacy of pressurization was checked twice daily. If an outflow tube failed to show pressurization at any time during the experiment, the animal was removed from the study.

The pressurized animals were monitored for food, water, outflow catheter patency, and general appearance twice daily for 1 week. After 1 week, the animals were sacrificed within a carbon dioxide chamber, and the left and right ventral bulla walls were exposed, leaving the inner and outer periosteum intact. Two bone fragments were removed (Fig 1). The first sample was from the ventral bulla wall, consisting of IM bone (sample I). The second sample was from the cochlear wall (EC bone) adjacent to the middle ear space (sample II). Specimens were immediately fixed by immersion in 2% glutaraldehyde and 2% paraformaldehyde in a 0.1 mol/L cacodylate buffer at pH 7.4. After fixation, the specimens were decalcified in a 0.1 mol/L disodium ethylenediaminetetraacetic acid with 4% glutaraldehyde solution for 1 week. Each specimen was then dehydrated in graded solutions of acetone, embedded in Epon-Araldite, and sectioned on a Reichert ultramicrotome to give triplicate 1-μm sections taken 100 μm apart. The sections were then stained with toluidine blue and basic fuchsin, double-blind labeled, and evaluated histomorphometrically.

Osteoclasts were identified histologically as multinucleate cells with ruffled borders abutting bone. Osteoblasts were identified as bone lining cells with single nuclei and homogeneous basophilic cytoplasm. Measurements of length of bone surface, percentage of the bone surface covered by osteoclasts, and osteoclast number were obtained from camera lucida drawings made with a computer-based histomorphometry system (VIAS, Ted Pella). For each parameter studied, the entire length of the sample bone surface was measured.

RESULTS

Pressurization of the middle ear to 10 mm Hg above atmospheric pressure led to an increase in osteoclast surface (Oc.S/BS) of the ventral bulla wall from 6.71% ± 1.41% at day 0 to 22.62% ± 3.34% at day 6. There was no significant increase in Oc.S/BS in the contralateral (unpressurized) control. In the same animals there was no increase in the Oc.S/BS in the adjacent EC bone of the cochlear wall. Of the 66 cochlear walls examined, only 1 osteoclast was found (see Table and Fig 2).

Comparisons were made between groups with a

INCREASE IN OSTEOCLAST SURFACE OF BULLAR AND COCHLEAR WALLS OF PRESSURIZED AND UNPRESSURIZED MIDDLE EARS

	Bulla Wall (%)		*Cochlear Wall (%)*	
Day	*Pressurized*	*Unpressurized*	*Pressurized*	*Unpressurized*
0	6.71 ± 1.41	5.41 ± 2.86	0.30 ± 0.61	0
1	4.15 ± 0.80	2.61 ± 1.30	0	0
2	9.98 ± 4.42	1.59 ± 1.18	0	0
4	12.10 ± 8.89	5.78 ± 1.32	0	0
6	22.62 ± 3.34	3.12 ± 2.02	0	0
8	14.96 ± 7.73	5.32 ± 4.47	0	0

factorial 2-way analysis of variance. Time and bone type were the independent variables, and Oc.S/BS was the dependent variable. There was a significant overall effect of time ($F_{1,52} = 104.1$, $p < .0001$), and IM bone had a significantly higher Oc.S/BS than did EC ($F_{1,52} = 5.87$, $p = .0003$). Further analysis revealed that there was a significant change in Oc.S/BS over time in the pressurized IM bone ($F_{2,52} = 11.1$, $p < .0001$), but there was no effect of time in the pressurized EC bone ($F_{2,52} = 0.004$, p = NS). The apparent decrease at day 8 was not statistically significant.

DISCUSSION

Endochondral bone is less subject to resorption by localized pressurization than IM bone. Resting osteoclast surface in the IM bone of the ventral bulla was 4% to 5% of that in previously published studies using this model.[1-3] In comparison, the EC bone of the medial bulla, the otic capsule, rarely had osteoclasts; only 1 osteoclast was seen in 52 specimens in this study. Pressurization to 10 mm Hg within the bulla results in an increase in osteoclasts within 4 days in IM bone but no osteoclast induction within the adjacent EC bone.

Endochondral bone differs from IM bone in several ways. Endochondral bone forms by the ossification of a hyaline cartilage model, and IM bone forms by the ossification of noncartilage connective tissue. Once formed, EC bone may differ fundamentally from IM bone. Smith and Abramson[4] compared IM and EC bone autografts and found that IM bone grafts actually increased in volume when transplanted into subcutaneous regions in rabbits, while EC grafts showed resorption. Similar studies replicated these results in rabbits[5] and in rabbits and monkeys.[6] Moskalewski et al[7] have shown that osteoblasts derived from EC bone (scapula) behave differently than those derived from IM bone (calvarium) when isolated and implanted into the posterior tibial muscle. Additionally, Kusiak et al[8] found further evidence that IM bone differs fundamentally from EC bone in that IM bone transplants were a stronger stimulus for revascularization than EC transplants; other investigators have not replicated this finding.[9]

Demineralized bone matrices derived from EC bone and IM bone were both shown to induce ossification when implanted subcutaneously,[10] but Scott and Hightower[11] later found that IM demineralized matrix, devoid of all possible contaminating EC tissue, did not induce ossification. This study suggested that EC bone matrix may differ fundamentally from IM bone matrix.

Although active osteoclasts have not been observed in the normal adult otic capsule, there is

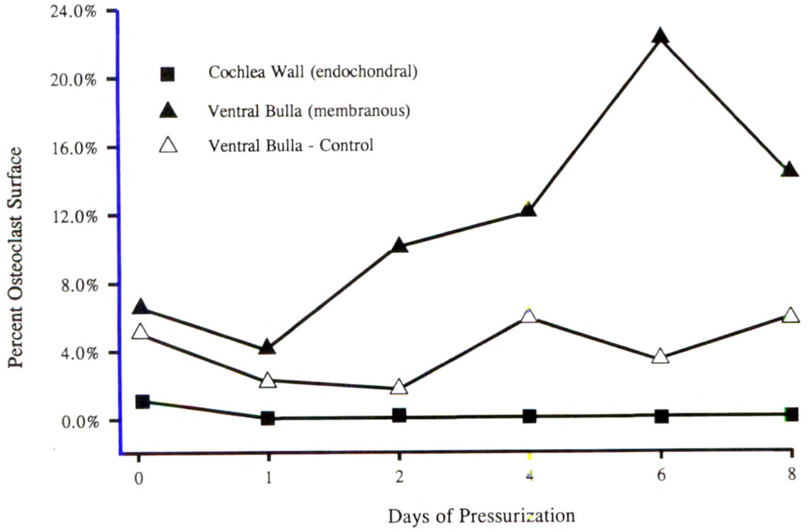

Fig 2. Change in osteoclast surface with time in each of samples. Points represent means.

evidence in animals[12,13] and in humans[14] of mineral exchange in the perivascular spaces and osteons of the otic capsule. Interestingly, the apparent mineral exchange, tetracycline labeling, is profoundly limited near the perilymphatic spaces.[12-14]

In the present study, the EC bone of the otic capsule showed significantly less induced resorption when compared to the identically treated IM bone of the ventral bulla. This finding provides support for the concept that EC bone differs fundamentally from IM bone in its resistance to induced resorption.

Another explanation of the findings of this study is based on the differences in gross structure of the EC bone sample and the IM bone sample. If strain is the signal for resorption, the thin (60 µm) IM bone of the ventral cochlea may be subjected to more strain by middle ear pressurization than the structurally buttressed EC bone of the cochlea (Fig 1). However, strains extant in the IM bone of the ventral bulla are probably less than 30 microstrains and may be too low to signal remodeling.

The findings in this study may be particularly germane to the understanding of the pathophysiology of otosclerosis. Otosclerosis is a localized activation of bone remodeling within the EC bone of the otic capsule. In this inherited disorder, osteoclastic resorption lacunae appear within the normal-appearing EC bone of the cochlear otic capsule. Resorption is followed by remodeling, resulting in a rather disorganized lesion of woven bone that may immobilize the stapes and affect the cochlea.[1] In the present study the EC bone of the otic capsule failed to respond to the induction of resorption that occurred in adjacent IM bone under identical experimental conditions, implying a fundamental difference in the 2 types of bone and their susceptibility to remodeling.

This study is also consistent with clinical observations in ears with cholesteatomas. The EC bone of the cochlea and otic capsule seems to be more resistant to resorption than adjacent structures that are composed of IM bone, such as the ear canal and the mastoid bone septae.

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CONGENITAL ANKYLOGLOSSIA WITH DEVIATION OF THE EPIGLOTTIS AND LARYNX: SYMPTOMS AND RESPIRATORY FUNCTION IN ADULTS

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We operated on 38 adult patients with congenital ankyloglossia with deviation of the epiglottis and larynx. The results were as follows. 1) Most patients had Angle's class III malocclusion, irregular alignment of the upper teeth, and high hard palate. 2) Fifty percent of the patients in our study population had obstructive respiratory failure. Their vital capacity increased significantly after the operation, but changes of forced expiratory volume in 1 second were not prominent. 3) Subjective symptoms of this disease were stiffness of the shoulders, a cold feeling in the extremities, an obstructed feeling in the throat, insomnia, fatigue, dry skin, irritability and/or anxiety, and nervousness. These improved postoperatively. 4) Objective symptoms included snoring, muscle cramps, difficulty in playing wind instruments, hoarseness, and incorrect articulation. The objective symptoms, except for incorrect articulation, improved postoperatively.

KEY WORDS — ankyloglossia, insomnia, larynx, occlusion, respiration.

INTRODUCTION

We have previously demonstrated that ankyloglossia is not only a forward dislocation of the tongue, but that it is also accompanied by an upper-forward dislocation of the epiglottis and larynx, and that newborn and suckling infants with this disease suffer from various degrees of dyspnea. Moreover, we reported on the symptoms and signs of congenital ankyloglossia in these children and, more important still, the relationship we think there is between this disease and sudden infant death syndrome.[1-6] In the past year we have treated several adult patients with ankyloglossia, and in this paper we report on the symptoms, signs, and state of respiratory function in adults with congenital ankyloglossia with deviation of the epiglottis and larynx (hereinafter called ankyloglossia).

MATERIALS AND METHODS

Thirty-eight patients who visited the Otorhinolaryngology Division of the Mukai Clinic between September 1989 and October 1990 were judged to have ankyloglossia. Ankyloglossia was diagnosed by the criteria we previously reported.[2-6] All of them underwent correction of the tongue, epiglottis, and larynx.[7,8] These structures were moved down and backward by cutting the front bundles of the genioglossus muscles and the same level of the lingual septum. We studied the patients' occlusion, the state of their larynges, and their respiratory function before and after the operation. We recorded their chief complaints and their comments after the operation.

Occlusion. We used Angle's classification for malocclusion, that is, class I for neutroclusion, class II for distoclusion, and class III for mesioclusion.[9]

Larynx. We observed patients' larynges with laryngofiberscopes (Machida ENT 30 II, Yokohama, Japan) via the nose and classified them into 2 categories based on the extent of the vocal folds observed during phonation. In a type A larynx, more than 50% of the vocal folds was observable during phonation. In type B, less than 50% of the vocal folds was seen because they were obscured by the aryepiglottic folds and corniculate and cuneiform tubercles (Fig 1).

Respiratory Function Tests. We measured the patients' vital capacity (VC) and forced expiratory volume in 1 second (FEV1) with an autospirometer (Autospiro 100, Minato Medical Instruments Co, Tokyo, Japan). The VC measured with this spirometer can be expressed in liters or volume percent according to the subject's age, sex, and height. For data standardization we expressed VC in volume percent. According to the measurement obtained, the spirometer automatically classified each patient as having normal, obstructive, or restrictive respiratory function, or a combination of both obstructive and restrictive. We compared the preoperative and postoperative conditions of the respiratory function by means of the spirometer.

Statistical Analysis. For statistical analysis of the

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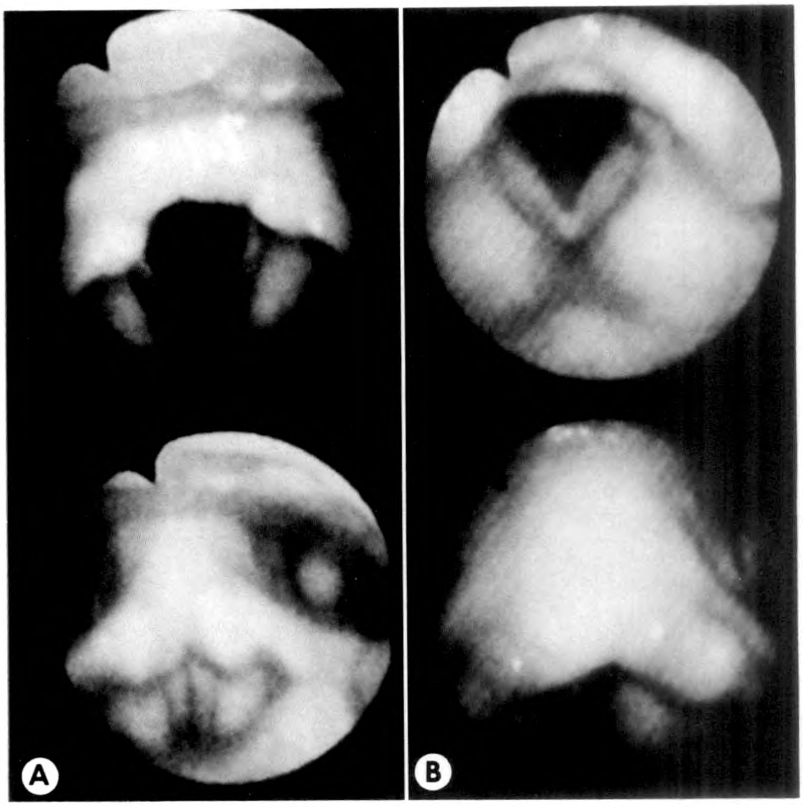

Fig 1. Laryngeal types. Top — during respiration, bottom — during phonation. A) Type A. B) Type B.

data we used McNemar's test, Student's paired *t* test, and the Stuart-Maxwell test.

RESULTS

Thirty-eight patients, 18 male and 20 female, were examined for this study. The age range was from 13 to 58 years old, with an average of 30 years and an SD of 9 years. One patient had undergone frenotomy as an infant.

Occlusion. Six patients (16%) had class I malocclusions; of these, 2 patients had received orthodontic treatment. The other 32 patients (84%) had class III malocclusions. There were no class II malocclusions. Besides malocclusion, all the patients had incisor and canine teeth that were large in comparison with the other teeth. Their upper teeth were not aligned (Fig 2), and they also had a cupolalike, high hard palate.

Larynx. Before the operation, 13 patients (34.2%) had a type A larynx, and 25 (65.8%) had a type B. After the operation, 8 patients showed a modification of the vocal folds' appearance during phonation, and the numbers changed to 21 (55.3%) for type A and 17 (44.7%) for type B. The statistical analyses (McNemar's test) revealed that the surgery had significantly affected the state of the larynges ($p < .02$).

Respiratory Function Test. Before the operation the average VC of the patients was 93.2%, with an SD of 18.8%, and the average FEV_1 was 60.6%, with an SD of 23.5%. The postoperative value for VC was 107.3%, with an SD of 18.4%, and that of FEV_1 was 66.7%, with an SD of 21.1% (Fig 3). The changes in VC were statistically significant by paired *t* test ($p < .0001$). Postoperative diagnoses by the Autospiro 500 were as follows: normal, from 8 cases before surgery to 20 cases; obstructive, from 19 to 15; restrictive, from 5 to 2; and combined, from 6 to 1 (Fig 4). Statistical analysis (Stuart-Maxwell test) revealed that surgery was effective in cases with obstructive and/or restrictive respiratory failure ($p < .005$).

Chief Complaints. Patients presented with the following complaints. We divided them into subjective and objective chief complaints. Subjective complaints were as follows: stiffness of the shoulder (21 cases), an obstructive feeling in the throat (20 cases), a cold feeling in the extremities (11 cases), insomnia and/or the feeling of not sleeping well (5 cases), and fatigue (2 cases). Objective complaints were as follows: incorrect articulation (5 cases), muscle cramps (2 cases), snoring (2 cases), difficulty in tonguing while playing the flute (1 case), and hoarseness (1 case; this patient had laryngeal polyps). The subjective complaints improved postoperatively. Of the objective complaints, snoring, difficulty in tonguing, muscle cramps, and hoarseness improved postoperatively, while problems with articulation were not affected.

Patients' Comments After Operation. The postoperative comments varied widely. Twenty of 38 pa-

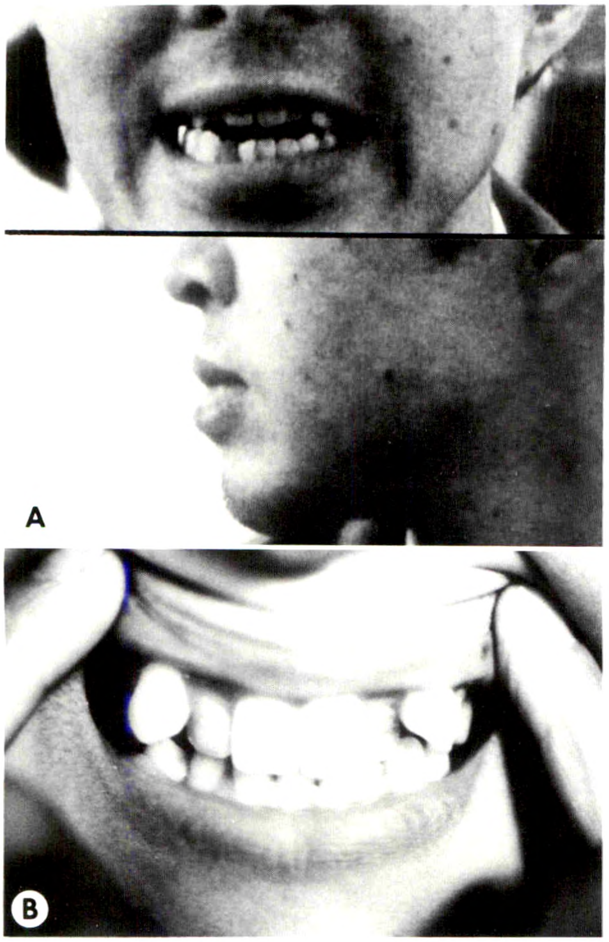

Fig 2. A) Observed Angle's class III mesioclusion. B) Malaligned upper teeth with large maxillary central incisors and canine teeth.

tients said they could breathe more easily. They could phonate better and for a longer time. Some felt their voices had become clearer (16 cases). Some could sleep better and/or felt rested in the morning (16 cases). Some felt their skin had turned brighter and healthier (11 cases). Some felt their throats to be more open (12 cases). Some had become calmer (2 cases). One patient had lost her feeling of anxiety and nervousness. According to their parents' observations, 2 patients who, before being operated on, showed signs of irritability did not have this sign anymore.

DISCUSSION

Occlusion. Eighty-four percent of the patients had Angle's class III malocclusion. Two patients out of 6 with class I malocclusion had orthodontic treatment. In addition, they had irregular tooth alignment, with large incisor and canine teeth and a cupolalike hard palate. On the basis of these observations we can say that ankyloglossia, along with an Angle's class III malocclusion, brings about irregular tooth alignment and the development of a high palate. In a report of a patient with ankyloglossia, Takasu[10] noted that the patient had irregular alignment of the upper teeth and especially of the canine teeth. Tuerk and Lubit,[11] on the other hand, reported that patients with ankyloglossia develop an infantile swallowing habit and have an open bite and Angle's class III malocclusion. They warned that the ankyloglossia greatly affects the shape and growth of the skull. We observed that some patients who could not bite with their molar teeth could do so after the operation. We also saw a patient who could not bite *sembei* (hard rice cakes) until she was operated on. We think this is probably because ankyloglossia interferes with the movements of the jaw. Besides the patient who said that she could not bend her back without discomfort, other patients also said that their back pain had disappeared after the operation; this leads us to think that ankyloglossia not only causes malocclusion but also brings about an imbalance of the spine. High hard palates were observed, also. We have already reported that infants with ankyloglossia have a high hard palate, and apparently it continues throughout life.

Larynx. According to our classification of the larynx, the A:B ratio before surgery was 13:25, and after the operation it was 21:17. This reveals that adults with ankyloglossia have the same deviation of the larynx as we previously observed in infants. After the operation infants could cry louder, and among the 38 patients reported here, 17 said their voices had become louder and clearer. These improvements of the voice were observed in those patients who went from type B to type A, as well as in those with type A who experienced further improvements. There was 1 patient with laryngeal polyps among the reported cases. We have seen 2 other patients with ankyloglossia who had laryngeal polyps (not included in these data). All of them underwent surgery to correct ankyloglossia, the epiglottis, and the larynx. Three months after surgery the polyps on the vocal folds had disappeared. Probably, when patients with ankyloglossia phonate, the vocal folds in the deviated larynx receive an excessive stress load and easily develop polyps.

Respiratory Function Test. The most prominent change in the respiratory function was an increase of the VC of the lung, an average of 14.1% that was statistically significant. The results were so amazing that at the beginning we could not believe what we were seeing. As we have already reported[1-3] in newborn and suckling infants, we observed that as soon as the operation finished the patients' skin turned pink and bright, and their features turned more vivid. We thought that these changes were due to the disappearance of the respiratory failure caused by the

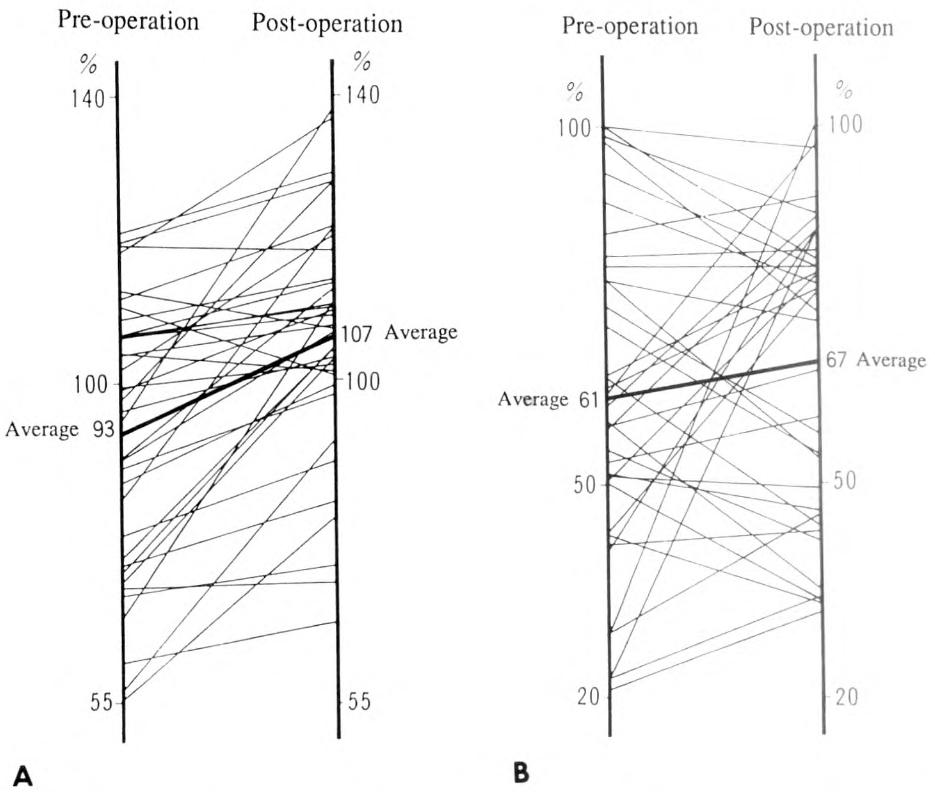

Fig 3. Preoperative and postoperative values for A) vital capacity and B) forced expiratory volume in 1 second.

increased upper airway resistance. But after seeing this increase in VC, we conclude instead that the most likely cause for the changes observed was an increased respiratory function due to the amplification of the VC after the surgery.[7,8]

There were patients who said that they could breathe better, and others who felt their throats were wider after the operation. The change in flow volume of FEV_1 was from 60.6% to 66.7%. This indicates that the upper airway flow increased when the deviation of the larynx was corrected, and consequently the patients felt these effects. Only 8 cases (21%) were diagnosed as normal by autospirometer. Among the rest, 19 cases (50%) were diagnosed as obstructive, 5 (13%) as restrictive, and 6 (16%) as combined. This means that respiratory function is restricted in a high percentage of adults with ankyloglossia. Obstructive respiratory failure was the most frequent diagnosis in our study population, and the cause of obstruction was probably increased upper airway resistance. This resistance resulted mainly from deviation of the larynx.

Improvement in Complaints. There were both subjective and objective complaints preoperatively. Of the subjective complaints, many improved. Of the objective complaints, only incorrect articulation was

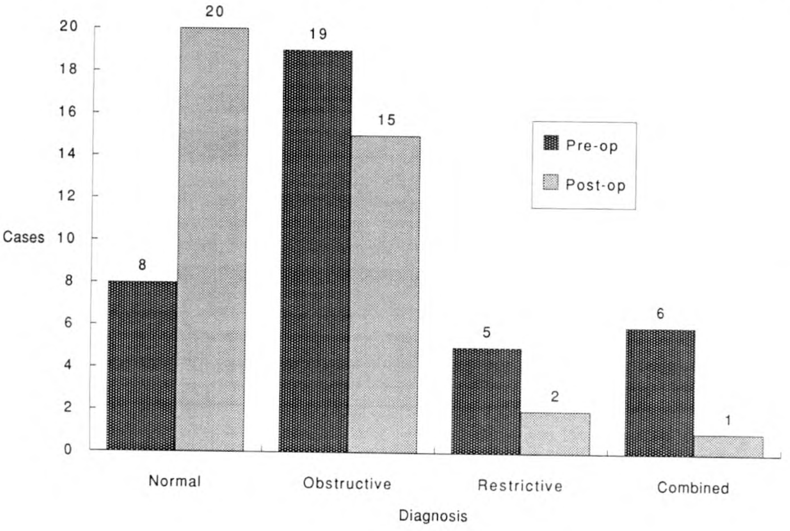

Fig 4. Changes in respiratory function as measured by Autospiro 500.

not affected; these patients did not realize their articulation was incorrect and therefore were not able to correct it.

Besides the 2 patients with noted muscle cramps, many other patients had muscle cramps during sleep. Muscle cramps occur frequently in pregnant women, in people with liver cirrhosis, and in old people, but the cause is unknown.[12-15] Our female patients had muscle cramps during pregnancy, too. We have reported that infants with ankyloglossia have pathologic muscle tonus.[1-6] We think ankyloglossia in adults also causes a state of pathologic muscle tonus that might develop as stiffness of the shoulders, muscle cramps, lumbago, snoring, fatigue, or respiratory failure.

There was a patient who could not play the flute well because tonguing was difficult. After surgery she could not only perform the tonguing well, but she also played with a more beautiful tone, with a narrowed glottic aperture.[16] Ankyloglossia with deviation of the epiglottis and larynx interferes with both tonguing and airflow in playing a wind instrument.

The patients' postoperative comments were, among others, that they had become calmer, that they had lost their feeling of anxiety and nervousness, that they were less irritable, and that their personalities had improved. The same tendency has also been observed in newborn, suckling, and postsuckling infants. It suggests that patients with ankyloglossia live in a state of stress.[17]

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MORPHOLOGY OF THE MEMBRANA LIMITANS

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The morphology of the membrana limitans (ML) was studied by light and electron microscopy to further understand its functional significance. The ML is a porous shield composed of a few to several layers of tightly packed fibrocytes that separate the perilymphatic space of the cochlea and saccule from that of the utricle and canal system. These fibrocytes are thinner on the side of the vestibule. They show many desmosomes and gap junctions and are interspersed with fibrils. The 3-dimensional reconstruction from celloidin sections shows that the ML, as it is viewed from the anterior side, is rectangular in shape, with various surface curvatures and an invagination toward the internal aperture of the vestibular aqueduct. Horseradish peroxidase, a tracer substance, placed on either side of the ML fails to pass to the opposite side. The ML and trabecular meshwork may be a factor contributing to differential sensitivity of cochlear and vestibular sensory cells in the presence of noxious substances.

KEY WORDS — cochlea, guinea pig, membrana limitans, perilymph, reconstruction, vestibule.

INTRODUCTION

The membrana limitans (ML) was described first by De Burlet[1] in 1920. Since then, very little attention was given to this structure until Tanaka[2] described it in 1975 in conjunction with the electron microscopy of the utricular walls in the guinea pig. Its functional role is little understood because of the lack of experimental studies. The purpose of the present study is to provide a visual representation of the ML from a 3-dimensional reconstruction and to give some insight into its physiologic significance based on a tracer study.

MATERIALS AND METHODS

In this study, the ML is defined as any cell layer or covering that separates the perilymphatic space of the vestibular cistern from that of the utricle and canal system. Thus, it includes not only a membranous layer attached to a trabecular meshwork, as described by De Burlet,[1] but also the similar cell layers covering the anterior surfaces of the utricle and lateral canal.

Guinea pigs were chosen for this study because we have a wealth of information on normal morphology and experimental data of this species from both light and electron microscopy. A total of 38 healthy Hartley guinea pigs were used in the entire study. Six guinea pig ears were fixed with Heidenhain-Susa, embedded in celloidin, and serially sectioned at 20 μm for light microscopy. Two guinea pig ears were fixed with Karnovsky's fixative and used for electron microscopy. Four guinea pig ears were fixed in Karnovsky's fixative, dissected, stained with toluidine blue, and examined under an operating microscope. In addition, celloidin sections from our temporal bone collection were examined.

In one of the celloidin-embedded specimens, 2 holes were drilled in the brain perpendicular to the cutting plane before sectioning. The centers of the holes were used as reference points for 3-dimensional reconstruction, a procedure that was described in a previous paper.[3] By means of all serial sections and with the aid of Macintosh software MacReco 3, the ML was reconstructed and its surface area was measured.

In order to understand the relationship between the ML and other structures, a semitransparent model of the ML was made with Epon, and models of the saccule and utricle were made with colored clay. For these purposes, photographs were made from all celloidin sections at magnification 50x. In constructing the Epon model, 4 photographs were printed from every section. The photographs were cut along the anterior surface of the ML. The posterior part of the ML was stacked in order according to the reference points. The thickness of 4 stacked prints was 1 mm. The cut surface of these stacked photographs was coated with thin Epon and hardened. For the clay model, a thin layer of clay was placed on top of the photographs and was cut along the margins of the structure. By using the reference points, these layers

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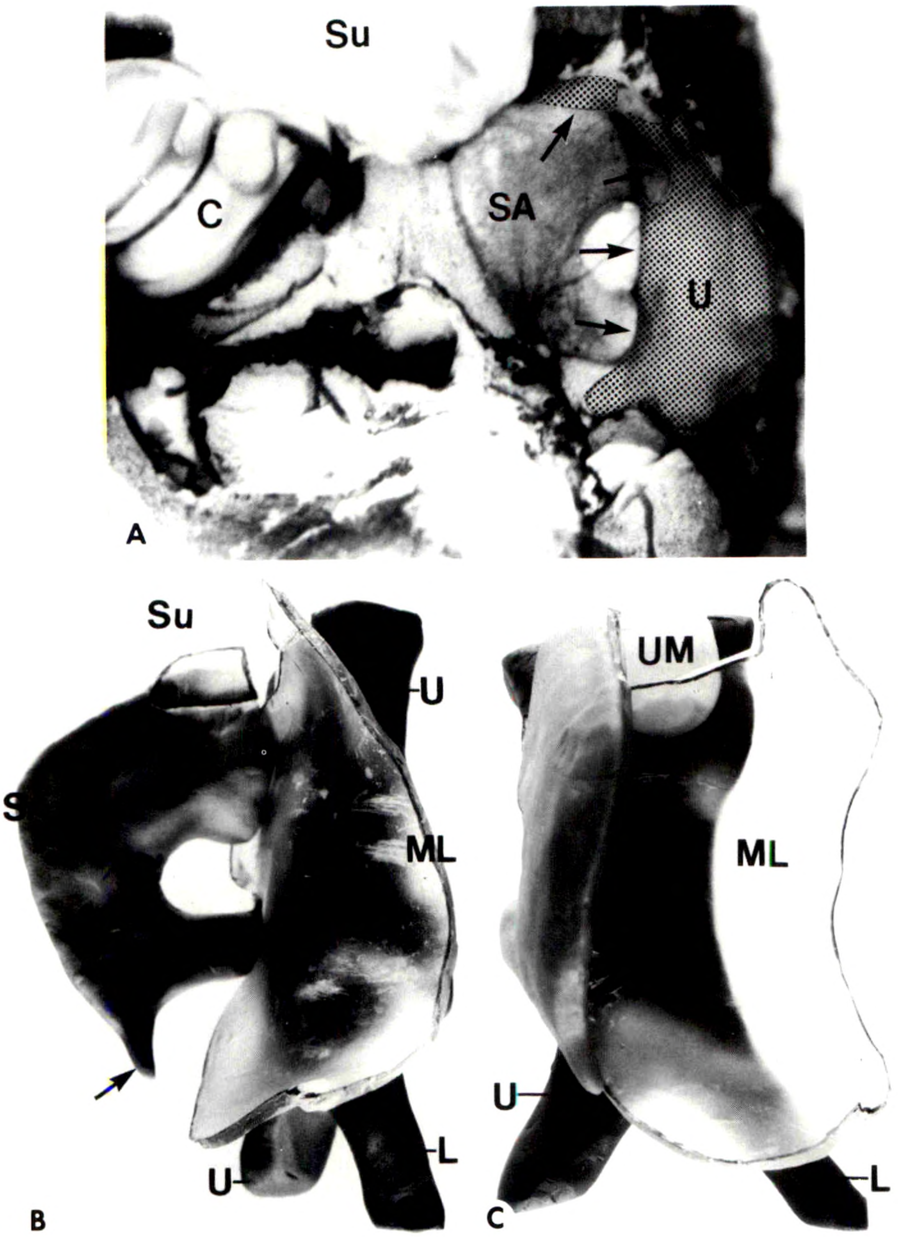

Fig 1. Three different views of membrana limitans (ML). A) Toluidine blue–stained ML (arrows) seen through operating microscope. Su — superior side, SA — saccule, U — utricle, C — cochlea. B,C) Three-dimensionally reconstructed ML (semiclear plastic). B) Viewed from cochlear side. L — lateral canal, arrow — ductus reuniens. C) Anterior-lateral view of rotated model seen from oval window side. UM — utricular macula.

were stacked and smoothed.

For the HRP experiments a total of 26 guinea pigs were used. Two different approaches were employed to determine the diffusion characteristics of the tracer without applying external force. An injection method was avoided, because our previous experience with gentamicin injection via pipets showed evidence of different drug distribution to the cochlea in comparison to the result obtained by the use of the diffusion technique.[4] In 10 ears, Gelfoam soaked with horseradish peroxidase (HRP) solution (Sigma HRP V1, 0.75 mg/mm^3) was placed gently on the oval window after removing the stapes. In 16 ears, Gelfoam soaked with HRP was placed over a fenestra of the lateral canal as described previously.[5] At the end of 1, 2, 4, 7, 10, 21, 30, and 90 days in the first group and 1, 2, 4, 5, 6, 7, 10, 21, and 30 days in the second group, the animals were sacrificed by intracardiac perfusion of Karnovsky's fixative. The ears were decalcified in disodium ethylenediaminetetraacetic acid and processed routinely for electron microscopy.

RESULTS

Three-dimensional reconstruction of the ML showed that it was rectangular in shape, and vertically long as viewed from the anterior (cochlear) side (Fig 1). The surface on the vestibule side was smooth and marked by various curvatures. The superior and inferior leaves were slightly bent toward the vestibule, and the elongated midsection showed a convexity in the same direction (Fig 1B,C). The surface area of the ML, including the overlapping part on the

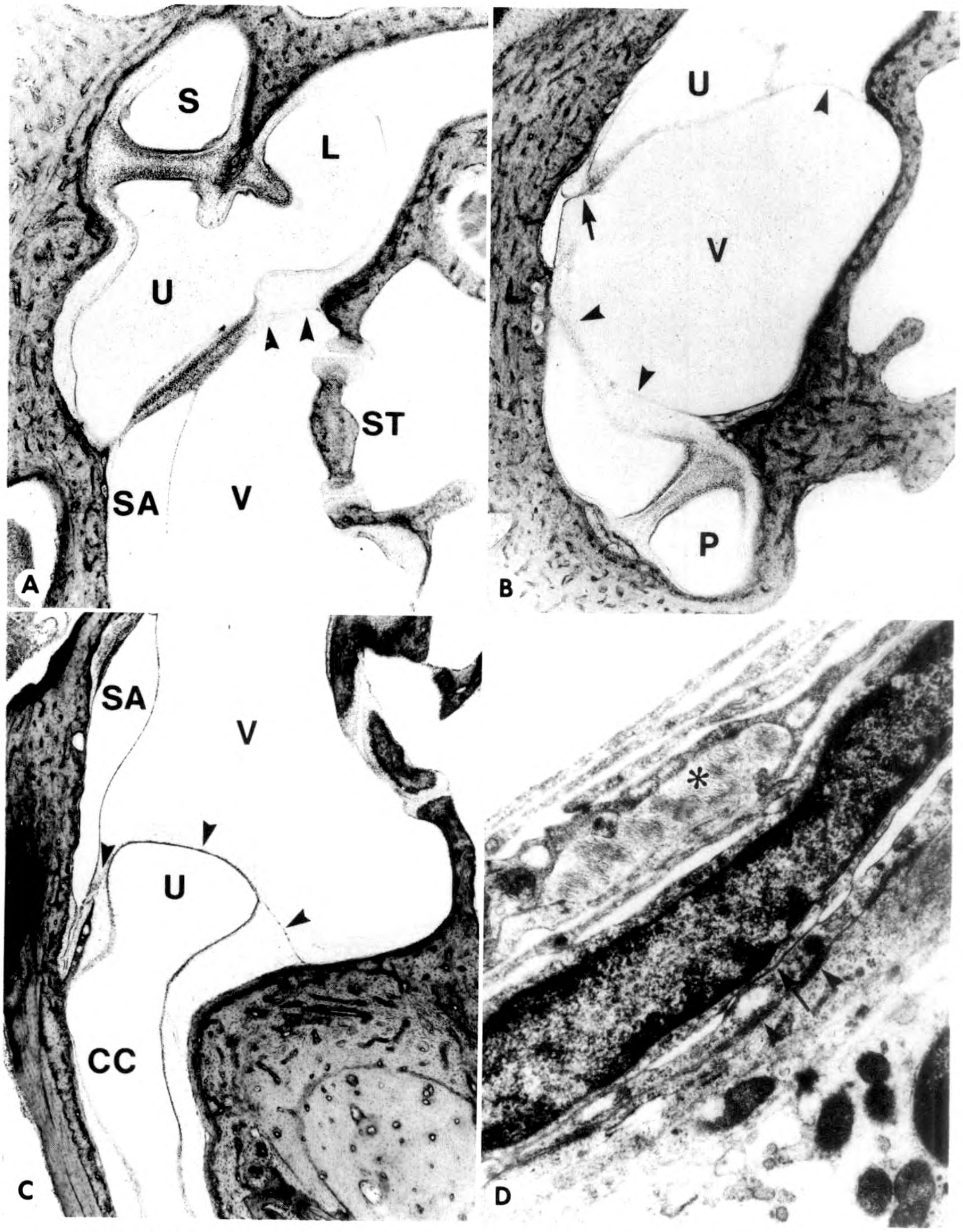

Fig 2. Light and electron microscopic views of membrana limitans (ML). A) Lateral ampulla (L) comes close to ML (arrowheads), which attaches to superior rim of oval window (radial section, original x4). Utricle (U) attaches to ML, but superior ampulla (S) does not come close to it. ST — stapes, SA — saccule, V — vestibule. B) Posterior ampulla (P) comes close to ML (arrowheads), which extends to utricle–endolymphatic valve area (arrow; radial section, original x4). C) Membrana limitans (arrowheads) invaginates toward vestibular aqueduct (horizontal section, original x4). Proteinaceous substances are shown on anterior surface of ML. CC — common crus. D) Electron micrograph of ML showing fibrocytes, gap junction (arrow), desmosomes (arrowheads), and fibrils (asterisk; original x6,000). Anterior surface of ML is on top.

saccule, was 4.18 mm^2. The reconstructed model (Fig 1B,C) was similar to that observed under the operating microscope (Fig 1A).

Light microscopy of serial sections revealed that superiorly the ML attached to the underside of the utricular macula and separated the lateral ampulla with its trabecular meshwork from the vestibule (Fig 2A,B). The ML did not come close to the superior ampulla and its canal. Near the utricular macula the

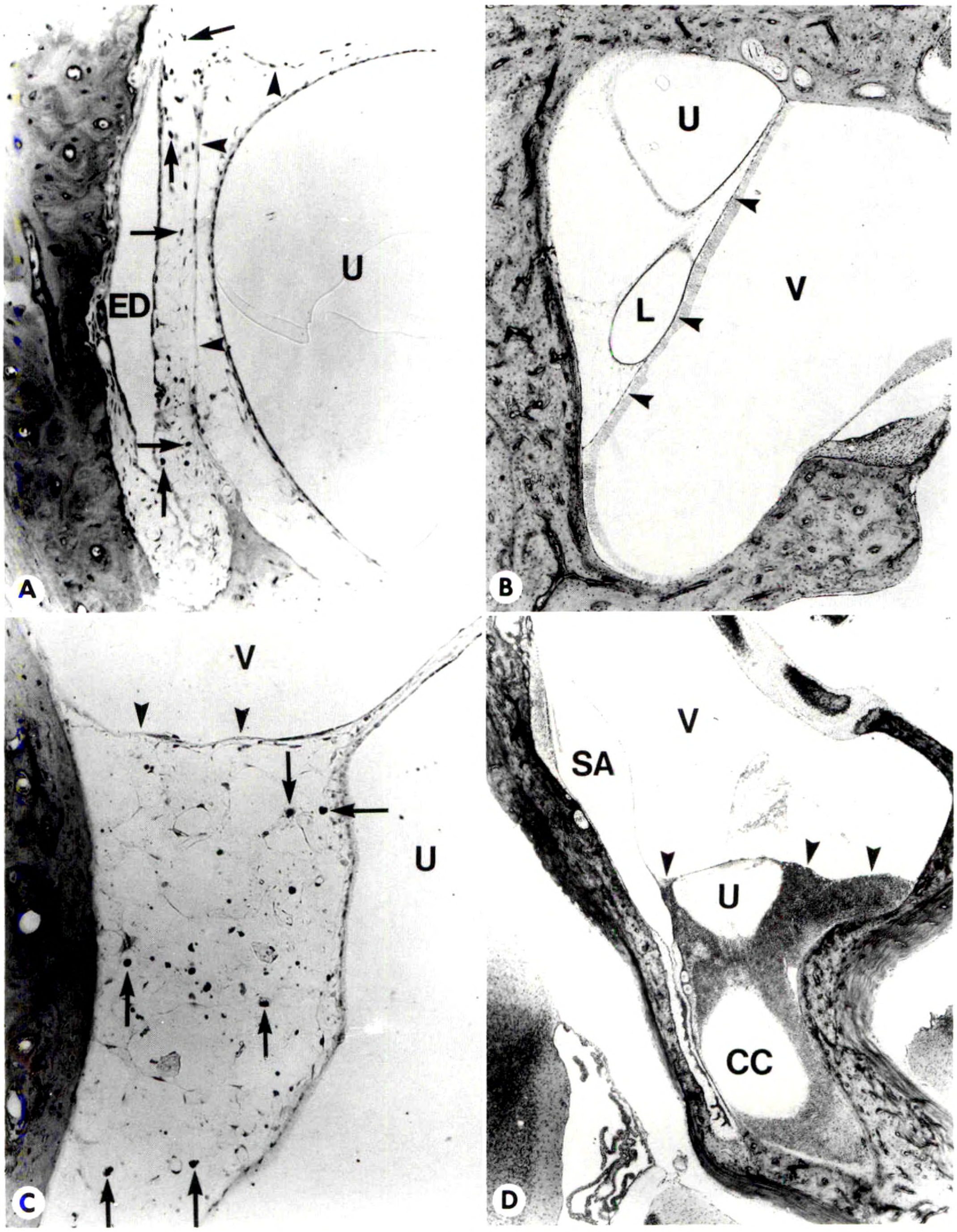

Fig 3. Membrana limitans (ML) acting as barrier. A) Membrana limitans invaginates and extends to bony rim of vestibular aqueduct (arrowheads; original x10). Horseradish peroxidase (HRP) infusion into vestibule (2 days earlier) shows that these tracers (arrows) are located at lateral side of aperture of endolymphatic duct (ED). U — utricle. B) Proteinaceous substances accumulated on anterior side of ML (arrowheads; horizontal section, original x4). L — lateral canal, V — vestibule. C) One week after HRP was introduced into perilymphatic space of lateral canal, tracer substances (arrows) are confined to posterior side of ML (arrowheads; original x20). D) Bleeding in perilymphatic space of U and common crus (CC; horizontal section, original x4). Blood cells are confined in perilymphatic space posterior to ML (arrowheads). SA — saccule.

ML extended partly over the saccular membrane. The ML made tight contacts with the entire length of the utricle in convex form toward the cochlea. It stretched from the utricle in both lateral and medial directions and attached to the endosteal layer (Fig 2A-C). Laterally, it came close to the superior rim of the oval window (Fig 2A), and medially, it was invaginated toward the internal aperture of the vestibular aqueduct (Fig 2B) and attached to its bony lip as a thin sheath (Fig 2C). Between this thin sheath and the endolymphatic duct was a loose fibrous network in which a proteinaceous substance often accumulated (Fig 2C). Inferiorly, the ML came into contact with the nonampullated end of the lateral canal wall and covered the trabecular meshwork of the posterior ampulla.

Electron microscopy revealed that the ML, which appeared as a single layer under a light microscope, was in fact composed of several layers of thin cytoplasmic extensions of the fibrocytes. Typically, these cell layers were thinner toward the vestibule than toward the posterior side. The overlapping cells of the ML were packed tightly and were interconnected by desmosomes and gap junctions. The intercellular spaces were generally small and were occupied by fibrils (Fig 2D). The fibrocytes contained small numbers of mitochondria and ribosomes, a poorly developed Golgi network, vacuoles, and vesiculated bodies; and a basal lumina was absent.

When Gelfoam soaked with HRP was applied to the oval window after removal of the stapes, the tracer substance was found on the anterior side of the ML 1 day after the operation. Two days after the operation, HRP was found between the lateral side of the endolymphatic duct and the ML (Fig 3A), but it was rarely found on the posterior side of the ML. The tracer substance was not obvious in the lumen of the endolymphatic sac at any survival time.

When HRP was applied in a similar manner to the perilymphatic space of the lateral semicircular canal, it was confined mostly to the posterior side of the ML. The HRP was found among fibrocytes up to 5 days after the operation, and thereafter only in macrophages (Fig 3C). In 1 case (6-day survival time), it was found in the subepithelial connective tissue of the endolymphatic duct.

A brief light microscopic examination of the ML in other species confirmed that the ML is present in the ears of the human, monkey, dog, cat, chinchilla, opossum, kangaroo rat, rat, bat, and mouse; but its extent appears to be variable. In adult human ears, the trabecular meshwork is greatly reduced. The ML is shown superiorly near the utricular macula and inferiorly at the utricle, close to the posterior ampulla and the nonampullated end of the lateral canal. However, in the zone between these 2 locations, where the lateral canal opens into the vestibule, the ML and trabecular meshwork are often absent, although they are present in the ears of the fetus and newborn.

DISCUSSION

Horseradish peroxidase placed in the vestibular cistern fails to pass through the ML. In other specimens in our temporal bone collection, it is not uncommon to find aggregates of eosinophilic precipitates limited to the anterior surface of the ML, as well as near the internal aperture of the vestibular aqueduct. Ferric compounds injected into the scala tympani through the round window membrane are reported to reach the perilymphatic space of the semicircular canals.[6] However, the result could be influenced by the injection of a large amount of tracer. Distribution of tracer substances in the perilymphatic space is most likely to depend on diffusion, rather than flow of fluid, the existence of which is doubtful. The morphological characteristics of the ML show that it is a loosely organized barrier and may prevent or delay substances from reaching the opposite side.

If toxic contamination of perilymph occurs in the cochlea, the sensory cells of the cochlea and saccular macula are more likely to be affected than those of the utricle and ampulla. Our unpublished study in which a small amount of gentamicin was injected into the scala tympani of the basal turn resulted in degeneration of the cochlear and saccular sensory cells without affecting other vestibular sense organs. The ML may not have prevented the diffusion of gentamicin to the other side, but it may have slowed down the diffusion process, keeping the drug concentration higher on the anterior side and thereby damaging sensory cells.

Our study of HRP infusion in the perilymphatic space of the lateral canal shows that these particles are mostly confined posterior to the ML. This observation is substantiated in an earlier study[7] in which perilymphatic hemorrhage around the vestibular sense organs was confined posterior to the ML, as shown in Fig 3D. It is worthwhile to note that the effects of gentamicin[5] and streptomycin[8] application to the lateral canal fenestra are toxic to all vestibular sensory cells located posterior to the ML, while hardly any effect was shown in cochlear sensory cells. Lesions of similar magnitude were shown among the different vestibular sense organs, with the exception of the saccular macula; this finding suggests that gentamicin and streptomycin are not necessarily accumulated at the ML, but are trapped in or adhered to the trabecular meshwork around the vestibular sensory cells. The ML with compact cell aggregates may have hindered further drug diffusion toward the cochlea. Thus, the ML serves as a partial barrier to movement of large or small molecular substances in either direction.

The major source of perilymph in the inner ear is considered to be the blood-labyrinth barrier.[9] The perilymph in the scala vestibuli and that in the scala tympani are different in composition and origin. This difference is attributed to the cells lining their perilymphatic spaces.[10] Similarly, the epithelial cell lining and connective tissue organization posterior to the ML are different from those of the cochlea, and the chemical composition and source of perilymph also could be different. The ML, trabecular meshwork, and capillaries may maintain homeostasis of perilymph separate from the cochlear system. Independent perilymph secretion and absorption may play a significant role in differential sensitivity of sensory cells to noxious substances present in perilymph anterior and posterior to the ML.

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SOURCES OF VARIABILITY IN POSTERIOR RHINOMANOMETRY

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Sources of variability in nasal airway resistance measured by posterior rhinomanometry were studied in 5 subjects tested on 5 different days and 56 subjects tested on 2 different days. On each day, a questionnaire on upper airway health and nasal symptoms was completed. The mean individual difference in nasal airway resistance between the 2 test days in the group of 56 subjects was 5.3% (SD 52.7%). Between-subject variability accounted for 74.9% and 72.5% of the total variability in the group of 5 and the group of 56 subjects, respectively. For the 5 subjects, by accounting for a change in upper airway symptoms or upper respiratory tract infection that occurred over the 5 test days, there was a significant decrease in the between-subject variability. The difference in sources of variation due to a change in upper airway symptoms was not seen in the group of 56 subjects. We conclude that the largest source of variability in nasal airway resistance is due to between-subject differences.

KEY WORDS — nasal airway resistance, rhinomanometry.

INTRODUCTION

Rhinomanometry has frequently been used in studies on upper airway physiology, although its clinical utility in individuals with nasal symptoms has not been established.[1,2] One limitation of rhinomanometry for clinical and research purposes has been the degree of variability in measurements of nasal airway resistance (NAR).[1,3] Potential sources of variability in the determination of NAR are the duration between measurements of NAR,[4,5] the particular flow or pressure values used to calculate NAR,[6] use of vasoactive nasal medications,[7,8] the presence of upper airway symptoms, physiologic changes,[9] and the age of the subject.[9]

In a study of ventilation patterns during different physical activities in a group of 56 individuals ranging in age from 7 to 72 years, we used a detailed airway health questionnaire to characterize subjects, and measured NAR on 2 occasions. To describe better the changes in NAR that occur over time, NAR was measured on 5 additional subjects on 5 different days. These data provided the opportunity to study the relations of subject characteristics, including nasal symptoms, with variability of NAR. We also assessed the number of curves needed per test session to estimate NAR at various degrees of precision in order to make recommendations on the number of curves needed for clinical or research purposes.

MATERIALS AND METHODS

Fifty-six subjects underwent posterior rhinomanometry on 2 different days separated by a mean of 10 days (range 1 to 38 days). The NAR was measured in another group of 5 subjects on 5 different days over the course of a week. A nasal history and symptom questionnaire was completed on each day. The experimental protocol was approved by the Institutional Review Boards of the University of New Mexico School of Medicine and the Albuquerque Veterans Administration Hospital.

Subjects. We recruited healthy volunteer subjects for the study through personal contacts, posters advertising the study on the medical school campus, and an announcement in a senior citizens' newspaper. The healthy subjects were selected to obtain equal representation of the sexes and a uniform age distribution. Inquiry was made to ensure that these subjects were in good health and free of heart or lung disease. Nasal symptoms or disorders were not considered in patient selection. No restrictions were placed on medication use prior to testing. We also recruited additional volunteer subjects with asthma, chronic obstructive pulmonary disease, and coronary artery disease from patient populations at the University of New Mexico Hospital and the Albuquerque Veterans Administration Medical Center. All volunteers gave informed consent and were paid for their participation.

From the Department of Medicine, Pulmonary and Critical Care Division, New Mexico Tumor Registry, Cancer Center, University of New Mexico Medical Center (all authors), and the Albuquerque Veterans Administration Medical Center (James, Chick), Albuquerque, New Mexico. Research described in this article was supported by National Institutes of Health National Research Service Award HL08446 (D.S.J.) and project number 89-7 from the Health Effects Institute (HEI), an organization jointly funded by the US Environmental Protection Agency (EPA; Assistance Agreement X812059) and automotive manufacturers. The contents of this article do not necessarily reflect the views of HEI, nor do they necessarily reflect the policies of EPA or automotive manufacturers.

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The 5 subjects who were studied on 5 different days were selected from laboratory personnel at one of the study institutions. The NAR had been measured in all 5 subjects on multiple occasions prior to the study.

Questionnaires. A questionnaire on baseline upper airway health with 24 questions was given on the first test day. A separate questionnaire with 13 questions on daily symptoms and medication use was given on both test days within 30 minutes of testing. Responses to the symptom questions were "absent" or "present." The questions on the upper airway questionnaire covered sinus problems or surgery; smoking history; nasal polyps; nasal discharge, congestion, or blockage at least once per week; broken nose; and rhinitis. Questions on the daily symptoms questionnaire addressed colds or respiratory infection in the past week; nasal congestion, discharge, or blockage on the day of testing; symptomatic hay fever on the day of testing; and medication use in the past week or the past 24 hours (oral or spray decongestants, antihistamines, corticosteroids, or cromolyn).

Rhinomanometry. All subjects were tested while sitting. The procedure was explained in detail and several trial runs were performed before recording began. Pressure and flow measurements were initially calibrated with a water manometer and rotameter. On each test day, pressure and flow readings were checked against a standard value with the manometer and rotameter. A nasal continuous positive airway pressure mask in 1 of 2 different sizes was held over the nose by the subject. This mask had a soft plastic rim that sealed tightly around the nose without distorting the nasal anatomy or affecting the rhinomanometry tracings in pilot studies. A 2-cm airflow tube attached to the mask was connected to a pneumotachometer (Erich Jaeger, Rockford, Ill). Pressure measurements were taken at a port on the nasal mask and through a 0.4-cm–inside diameter plastic tube held as far back in the mouth as the subject tolerated. Nasal airflow and the nose-mouth pressure difference were plotted on an x-y recorder.[10]

Occlusion of the oral tube by the subject and air leaks around the seal of the nasal mask were readily apparent on the tracing. Subjects were asked to take a slow tidal breath while the mask and oral tube were in place. A minimum of 5 curves from each subject were recorded per test day; 8 subjects were asked to do 10 curves per test day. Subjects were asked to return for their second test within the following 6 weeks. The 5 subjects in the smaller test group had 5 curves recorded on different days over a 7-day period. Subjects were studied at the same time on each of the 5 test days.

If looping of the tracing occurred, only the initial phase of the inspiratory curve was used in measurements. Pressure measurements were read by eye from the inspiratory portion of the curves at flow increments of 0.048 L/s (2 mm on the tracing). The origin was determined by the point at which the curve crossed the zero flow line. Nasal resistance was calculated at a flow of 0.25 L/s. In the pilot study, the majority of curves had a maximum flow of at least 0.25 L/s. On completion of the entire study, 96% of all curves reached a maximum flow of at least 0.25 L/s.

Analysis. To calculate NAR, Rohrer's equation, $P = K_1 \times V + K_2 \times V^2$, was transformed to $P/V = K_1 + K_2 \times V$, where P is pressure (centimeters of water), V is flow (liters per second), and K_1 and K_2 are constants.[10,11] Linear regression analysis was used on the transformed equation to determine K_1 and K_2. For each curve, NAR was calculated at a flow of 0.25 L/s by using the regression coefficients, K_1 and K_2.

The natural log of NAR and standard deviation (SD) of NAR were used for all statistical analysis because the log transformed values had an approximate normal distribution and log transformation standardized the variance. Because NAR and the SD of NAR have asymmetric distributions, the median and interquartile range (IQR) are given as summary values rather than the mean and SD, unless noted otherwise. The IQR is the difference between the third and first quartiles. A p value less than .05 was considered significant.

To compare NAR and SD of NAR between day 1 and day 2, the mean of the log NAR or SD of NAR was plotted against the difference in log NAR or SD of NAR on day 1 and day 2.[12] Paired *t* tests were used for comparison of NAR and SD of NAR between test day 1 and test day 2. Differences in between-group mean NAR and SD of NAR were examined by using two sample *t* tests and analysis of variance. Variations among subjects, between days, and within days were examined with a 2-factor random effects model that included main effects of subjects, days, and the subject-day interaction.[4,13,14] Because subjects and days were considered to be random effects, the F ratio for each main effect was calculated by using the interaction mean square in the denominator.[14]

To determine the optimal number of tracings to perform per session, the standard error for each individual for different possible numbers of curves was calculated from the SD of NAR divided by the

TABLE 1. NASAL AIRWAY RESISTANCE FOR DIFFERENT SUBJECT CHARACTERISTICS FOR BOTH TEST DAYS IN GROUP WITH 56 SUBJECTS

	N	*Median*	*SD*
Sex			
Male	32	1.13 (0.90)	0.22 (0.30)
Female	24	1.52 (1.16)	0.28 (0.24)
Age			
7-13	11	1.73 (2.31)	0.43 (0.77)
16-60	30	1.15 (0.79)	0.22 (0.21)
61-72	15	0.90 (1.59)*	0.18 (0.34)†
Cardiopulmonary disorder			
None	41	1.18 (0.88)	0.25 (0.22)
Asthma	5	1.48 (1.73)	0.29 (0.36)
COPD	5	1.13 (0.39)	0.31 (0.49)
CAD	5	0.78 (0.48)	0.15 (0.04)

Data are in centimeters of water per liter per second. Values in parentheses are interquartile range.

COPD — chronic obstructive lung disease, CAD — coronary artery disease.

*p = .002 (analysis of variance on log transformed nasal airway resistance and SD of nasal airway resistance).

†p = .03 (analysis of variance on log transformed nasal airway resistance and SD of nasal airway resistance).

TABLE 2. NASAL AIRWAY RESISTANCE FOR TEST DAY 1 AND TEST DAY 2 FOR GROUP WITH 56 SUBJECTS

	NAR		*SD of NAR*	
	Day 1	*Day 2*	*Day 1*	*Day 2*
Mean	1.46	1.61	0.41	0.35
SD	1.09	1.54	0.47	0.46
Median	1.09	1.15	0.23	0.22
IQR	0.94	1.03	0.23	0.25
Minimum	0.30	0.24	0.02	0.02
Maximum	5.41	9.62	2.17	2.96

Data are centimeters of water per liter per second.

NAR — nasal airway resistance, IQR — interquartile range.

square root of the number of curves (2 to 10). By using the 95% confidence intervals for the mean resistance obtained from the product of each individual's standard error and the *t* statistic, we determined the number of curves with confidence interval lengths less than 0.4, 0.6, and 0.8 cm H2O/L per second for each possible number of curves performed per session. We used SAS software (Statistical Analysis Systems, Cary, NC) for all analyses.

RESULTS

Posterior rhinomanometry was used to measure NAR in a group of 56 subjects on 2 different days. Subject characteristics are given in Table 1. The mean age of subjects was 39 years (range 7 to 72 years). Descriptive statistics for NAR on test day 1 and test day 2 are given in Table 2. The median NAR for all subjects from both test days was 1.15 cm H2O/L per second (IQR 0.96 cm H2O/L per second). No statistically significant differences were found in NAR by responses to any of the upper airway health or daily symptom questions on a single test day.

To examine better the day-to-day variability in NAR, a smaller group of 5 additional subjects, 1 woman and 4 men, was studied on 5 different days over a week. The mean age of this group was 27 years (range 22 to 31 years). To summarize the results from the daily upper airway symptom questionnaire, a symptom score was calculated from the sum of the presence (1) or absence (0) of upper respiratory tract infection in the past week, nasal congestion or blockage, or nasal discharge. The maximum possible score was 3; the minimum score was 0. Figure 1 displays the NAR and daily symptom score for the 5 subjects on each of the 5 test days. The change in the symptom score followed the NAR change in 4 of 5 subjects, and subjects with symptoms tended to have higher NAR values than subjects without symptoms (Fig 1).

Variability in NAR was then examined. One source of variability was the magnitude of the NAR value. As NAR increased, the SD of NAR also increased as described in the linear regression equation: $\text{SD of NAR} = 0.31 \times \text{NAR} - 0.08, r = -.85, p = .0001$. With natural log transformation, there was no statistically significant association between NAR and SD of NAR ($p = .87$). Log transformation of NAR and SD of NAR normalized the variance and created a normal distribution that was used for all statistical analyses.

The within-subject variability was assessed by examining changes in NAR between the 2 test days. The mean individual difference between NAR on test day 1 and that on test day 2 was 0.15 cm H2O/L per second (SD 1.23). The mean individual percent change in NAR between test day 1 and test day 2 was 5.3% (SD 52.7%). The between–test day repeatability of NAR is shown in Fig 2A. Ideally, the mean difference between the 2 test days would be 0, and 95% of the differences would be less than 2 SD from the mean.[12] Three of 56 NAR values (3.6%) were greater than 2 SD from the mean log NAR difference (Fig 2A).

Between-subject variability was examined by determining the difference in the SD of NAR on a single day between different subject characteristic groups. There was a significant decrease in the SD of log NAR with increasing age (Table 1). However, there were no significant differences in NAR or SD of NAR between levels of other subject characteristics or between subjects classified by responses to any of the upper airway health or symptom questions on the 2 test days.

Sources of variability that could have resulted

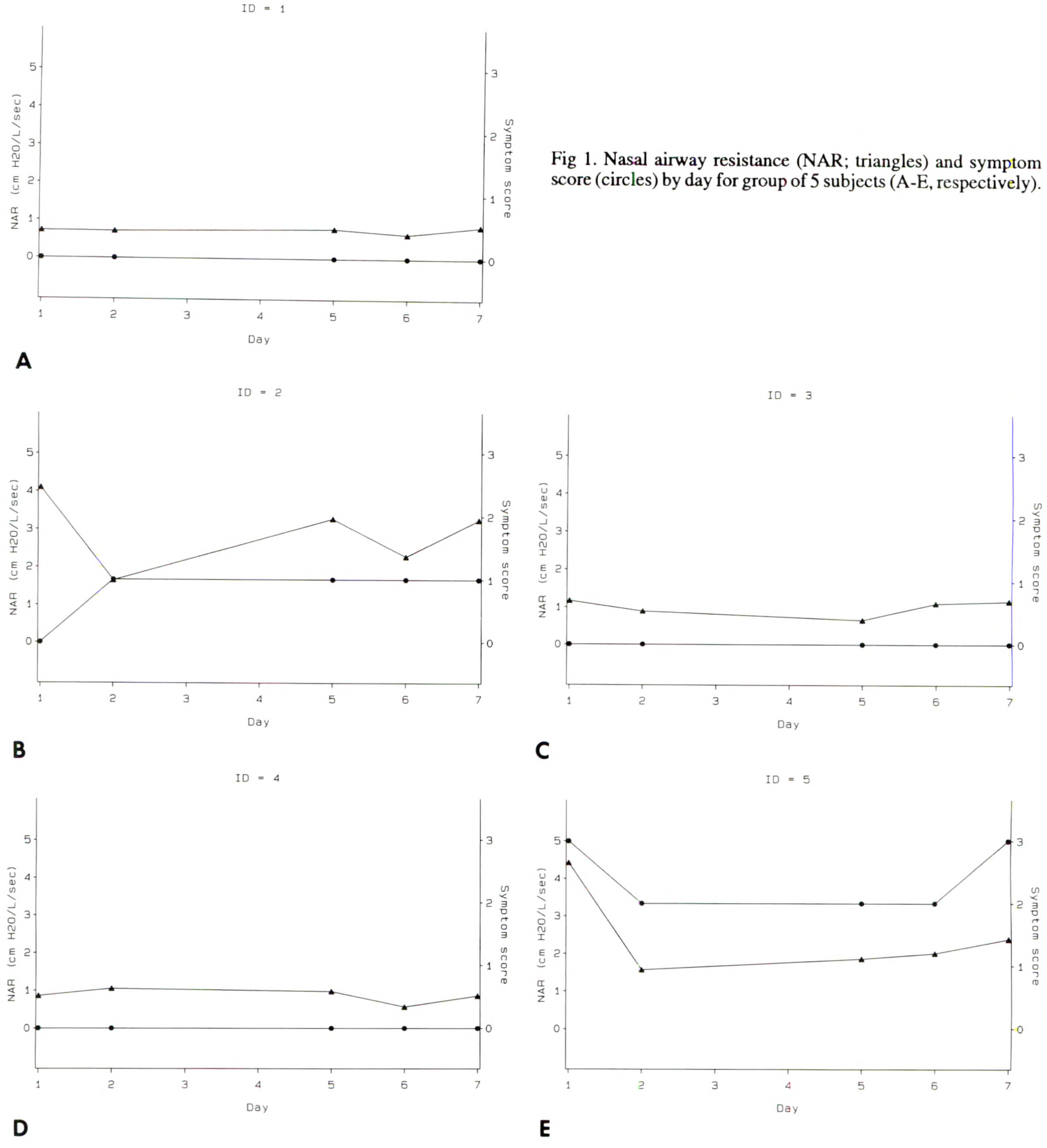

Fig 1. Nasal airway resistance (NAR; triangles) and symptom score (circles) by day for group of 5 subjects (A-E, respectively).

from the technique of NAR measurement were also examined. Figure 2B shows the difference in log SD of NAR between test day 1 and test day 2. The mean percent difference in SD of NAR was 8.28%; this was not significantly different from 0 (p = .18). This suggests that individuals did not improve in their ability to perform the test between the 2 test days. No trend was observed in the SD of NAR over the time course of the study as seen in the linear regression model: SD of NAR = 0.001 x day of study – 5.6, r = .10, p = .48. This suggests that there were no significant changes in the efficiency of instruction to individuals as the study progressed.

Nasal medications were infrequently used by subjects. Oral decongestants were used by 4.5% and 9.8% of the 56 subjects within 24 hours and 7 days, respectively, of testing. Nasal decongestant sprays were used by 4.5% and 5.4% of the subjects within 24 hours and 7 days, respectively, of testing. No subjects used nasal corticosteroids or nasal cromolyn. The NAR and SD of NAR were not significantly different between subjects who did and did not use

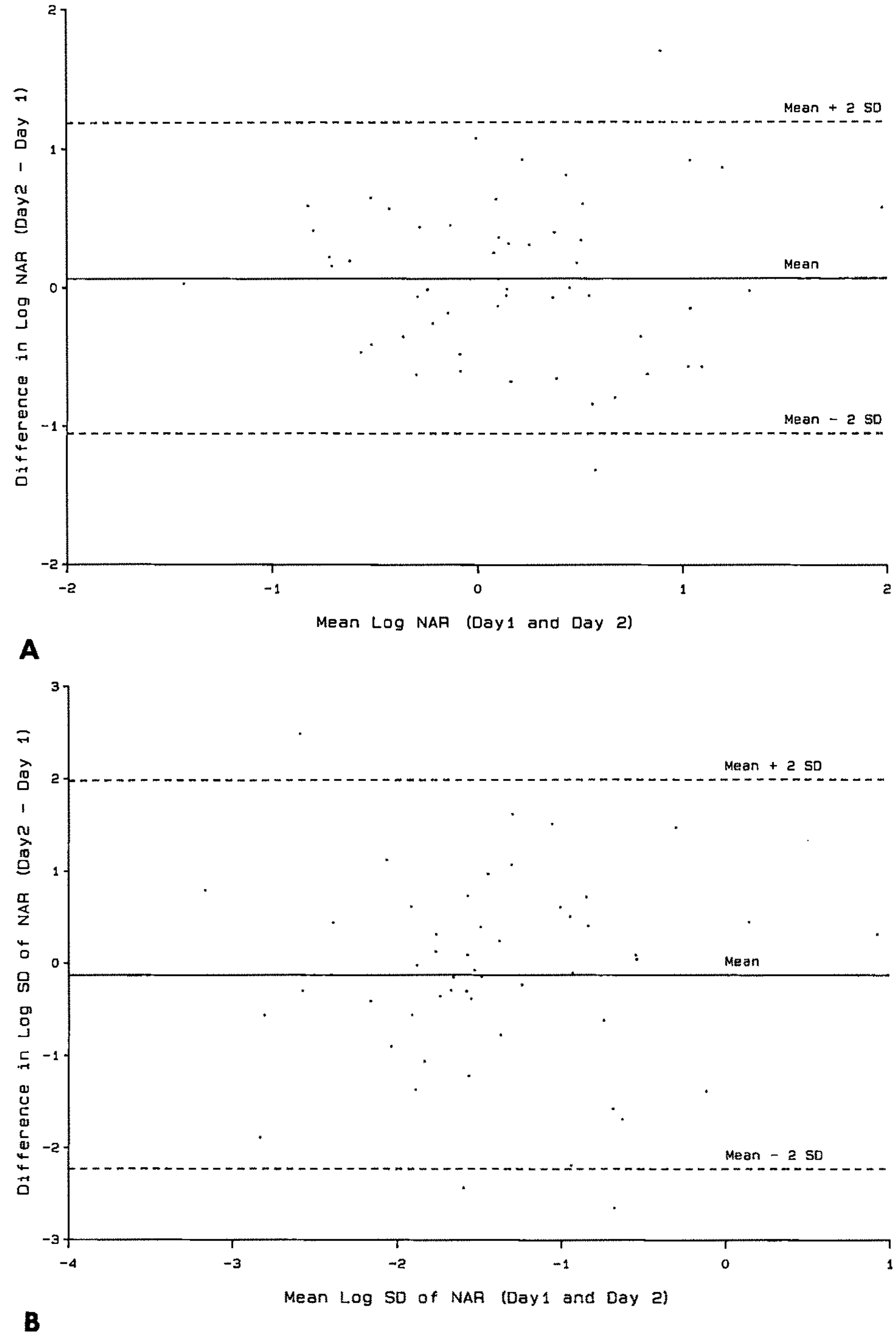

Fig 2. Difference in A) nasal airway resistance (NAR) and B) SD of NAR between day 1 and day 2 against mean NAR or SD of NAR for group of 56 subjects (log transformed data).

any oral or spray decongestant.

The relative magnitudes of the components of variation in NAR were examined by analysis of variance methods (Table 3). In the group of 5 subjects, between-subject, between-day, subject-day interaction, and error terms accounted for 74.9%, 5.9%, 10.3%, and 8.3% of the total variance in log NAR. In the group of 56 subjects, between-subject, between-day, subject-day interaction, and error terms accounted for 72.5%, 0.01%, 14.8%, and 12.6% of the total variance in log NAR. To examine the importance of upper airway symptoms on the between-subject variability, the daily symptom score was used to categorize subjects into groups with and without a change in symptoms between test days. In the group of 5 subjects, for subjects with a change in their daily symptom score, between-subject, between-day, subject-day interaction, and error terms accounted for 6.3%, 63.9%, 2.0%, and 22.5%, respectively, of the total variance in NAR. A smaller percentage of the total variance was from the between-subject differ-

TABLE 3. ANALYSIS OF VARIANCE OF NAR IN GROUP OF 5 SUBJECTS AND GROUP OF 56 SUBJECTS

Source of Variation		*All Subjects*	*Subjects by Symptom Group* *No Change in Symptoms*	*Change in Symptoms*
5 Subjects, 5 days				
Subject	*df*	4	2	1
	MS	9.45*	0.74	0.50
Day	*df*	4	4	4
	MS	0.74	0.12	1.29†
Subject* Day	*df*	16	8	4
	MS	0.34	0.28*	0.15†
Error	*df*	100	60	40
	MS	0.04	0.04	0.04
56 Subjects, 2 days				
Subject	*df*	55	29	25
	MS	3.72*	3.36*	4.27*
Day	*df*	1	1	1
	MS	0.15	0.07	0.07
Subject* Day	*df*	55	29	25
	MS	0.76*	0.84*	0.70*
Error	*df*	492	227	263
	MS	0.07	0.08	0.07

NAR — nasal airway resistance, *df* — degrees of freedom, MS — mean square.

*$p < .001$ (F test).

†$p < .05$ (F test).

ences in the group with a change in the daily symptom score when compared to the group without a change in symptom score (6.2% versus 22.6%). In the group of 56 subjects, the between-subject variability was approximately the same in the groups with and without a change in the daily symptom score (70.0% versus 74.9%).

For both research and clinical purposes, it may be necessary to measure NAR to a specified degree of precision. Based on the within-subject variation in the group of 56 volunteers, the precision achieved by performing different numbers of curves per test session is shown in Fig 3. To improve the precision of the NAR estimate, more curves per session are required (Fig 3). For example, to estimate NAR to within ±0.4 cm H_2O/L per second (a 95% confidence interval length of 0.8 cm H_2O/L per second), 5 curves would adequately estimate NAR 75% of the time and 10 curves would adequately estimate NAR 83% of the time (upper curve in Fig 3).

DISCUSSION

The use of rhinomanometry in the clinical setting, to assess the severity of an individual's nasal symptoms or for research on upper airway physiology, for example, has been limited by the lack of a consistent association between NAR and relevant clinical and physiologic variables.[2,15,16] The degree of variability in NAR measurements may explain this pattern of limited and inconsistent use. The methods used to measure and calculate NAR, the presence of upper airway disease, the nasal cycle or movement of the soft palate, and symptom status may affect the level of NAR and its variability.

The median NAR reported in this study, 1.15 cm H_2O/L per second, is in the range of values obtained in other studies using posterior rhinomanometry: 1.04 to 2.48 cm H_2O/L per second.[4,6,7,10,17] Each of these other studies used different flow or pressure values to determine NAR. The median SD of NAR in our study was 0.23 cm H_2O/L per second, similar to values in other studies using similar techniques: 0.20 to 0.42 cm H_2O/L per second.[4,6,7,10,17] Automated rather than manual curve analysis might have decreased the variability introduced by the calculation of NAR. Differences in the SD of NAR can also occur from varying values of pressure or flow at which NAR is calculated. The SD of NAR has been found to increase at higher flow values in another study.[6]

Most of the 56 individuals had only a small difference in NAR between test day 1 and test day 2 (Fig 2A). In 96.4% of individuals, the difference in NAR between test day 1 and test day 2 was within 2 SD of the mean (Fig 2A). In contrast, for the 5-subject and 56-subject groups, the between-subject variability in NAR on each of the test days was large, accounting for 74.9% and 72.5%, respectively, of the total variance (Table 3). Using similar technique and analysis of variance, Dvoracek et al[4] also found that the largest proportion of total variance was due to between-subject variability. Thus, for most individuals, NAR measured by posterior rhinomanometry is a reproducible measurement.

In a study examining the short-term variability in NAR, Hasegawa et al[18] used posterior rhinomanometry to determine NAR in 50 subjects. The median NAR was 1.53 H_2O/L per second (IQR 0.78 cm H_2O/L per second), similar to the values found in the current study. Hasegawa et al also studied the changes in NAR, comparing measurements made 15 minutes apart over 7 hours. The median change in NAR over this period was 13.4% (IQR 18.6%), with a range from 0% to 400%. Using the 95th percentile of the change in NAR, Hasegawa et al concluded that a "change greater than 53% suggests a significant change that cannot be attributed to physiologic variability."[18(p70)] The researchers did not attempt to document changes in nasal symptoms over the study period.

The use of rhinomanometry in clinical settings has

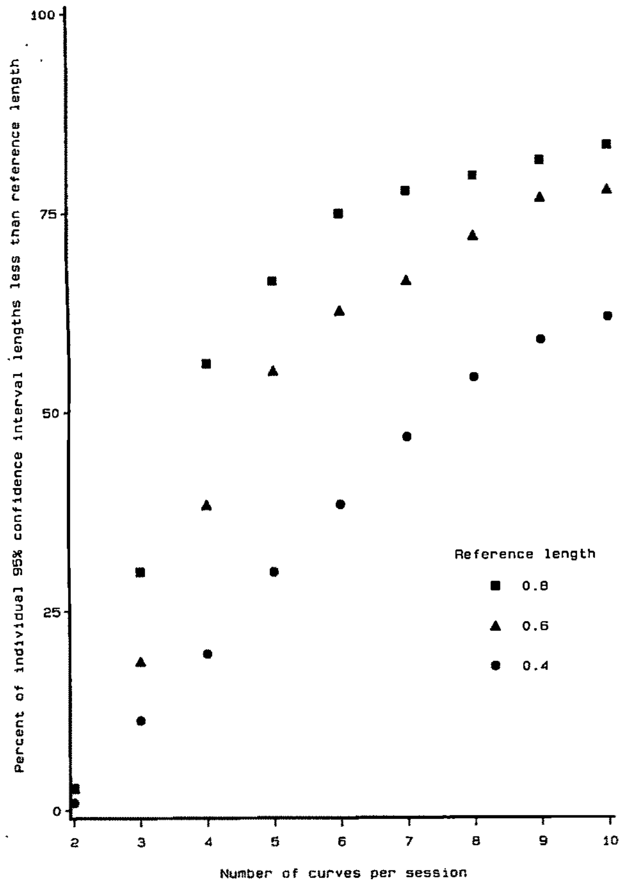

Fig 3. Estimated percentage of curves with nasal airway resistance estimated within different 95% confidence interval lengths by number of curves performed per test session.

been further limited by the relatively modest correlations of NAR with subjective symptoms.[2,7,8] Gleeson et al[8] compared posterior rhinomanometry with subjective assessment of nasal patency and found a moderate correlation ($r = .47$). McCaffrey and Kern[2] used anterior rhinomanometry to study 974 subjects, and found that moderate and severe subjective symptoms of nasal obstruction were associated with higher values of NAR. Symptoms of nasal obstruction occurred primarily at NAR values greater than 3 cm H_2O/L per second.[2] Applying a similar cutoff value of 3 cm H_2O/L per second to the subjects does not adequately separate our subjects with upper airway symptoms or disorders from normal subjects. Cole et al[7] found higher NAR in subjects with rhinitis or fixed obstruction compared to normal subjects. The current study using 37 questions on upper airway health and symptoms found that the median NAR for subjects with a symptom was frequently larger than the median NAR when the symptom was absent; however, because of the large between-subject variability these differences were not statistically significant. In the group of 5 subjects, changes in an index of nasal symptoms and upper respiratory tract infection followed changes in NAR in 4 of 5 subjects (Fig 1). For subjects developing or resolving a particular nasal symptom between the 2 test days, a larger percentage of the variance in NAR was due to the between-day effect compared to subjects without changes in symptoms. The effect of a change in symptoms on the percentage of variance in NAR was not found in the group of 56 subjects. More subjects with a change in nasal symptoms would be required for adequate statistical power to detect clinically important changes in NAR associated with acquiring or losing nasal symptoms.

The number of curves performed on each individual during a test session could affect the precision of the estimate of NAR; with a larger number of curves, the precision would increase. Using estimates of the variance from published reports,[6,7] we wanted to estimate NAR to within ±0.4 cm H_2O/L per second. Ten curves were initially found to be adequate to achieve the predetermined variability. Analysis of the data after 8 individuals had been tested revealed that 5 curves would estimate NAR within ±0.4 cm H_2O/L per second more than 50% of the time. Five curves per session were used for the remainder of the study. When all subjects were examined upon completion of the study, 65% of mean NARs were estimated to within ±0.4 cm H_2O/L per second. Ten curves per test session would decrease the within-subject variability even further and could be easily performed in a short amount of time. If 10 curves per test session were used, 62% of the mean NAR values would be estimated to within ±0.2 cm H_2O/L per second (Fig 2).

One criticism of posterior rhinomanometry has been the inability of 17% to 30% of individuals to perform the procedure.[3,8] All subjects in this study were able to perform at least 1 adequate curve. In only 16 of 112 sessions (14%) were individuals unable to perform the required 5 or 10 curves per test session.

Studies on the upper airway have been hampered by the lack of a reproducible and accurate measure of resistance to airflow that correlates with clinical and physiologic variables. In this study, we have examined factors affecting the variability in NAR measured by posterior rhinomanometry. We found that between-subject differences are the largest source of variability in NAR; a change in nasal symptoms between NAR measurements in some subjects may be a significant source of variability; and 10 curves per testing session is an adequate number to estimate the NAR to within ±0.2 cm H_2O/L per second. Other factors contributing to the differences in NAR between individuals remain to be determined.

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MUCOSAL FINE STRUCTURE IN EXPERIMENTAL SINUSITIS

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Rabbit maxillary sinuses were inoculated with *Streptococcus pneumoniae* and *Bacteroides fragilis*, and the histologic response in the sinus mucosa was observed over a 12-week period. An increased height of the cylindric cells and hyperplasia of the basal cells were frequent findings irrespective of the pathogen inoculated. The disease was found to influence the character of the secretory product from epithelial secretory cells and to degranulate the subepithelial glands. Ciliary loss was a transitional finding. A reduction in the number of mitochondria, the occurrence of deformed short microvilli, and cytoplasmic blebbing were seen in the cells devoid of normal cilia. It is inferred from this study that pneumococcal sinusitis in rabbits is a self-limiting process, and the mucosal sequelae of the acute infection are persisting goblet cells, slight focal fibrosis, and edema. Inoculation with *B fragilis* produces a chronic inflammatory process, with infiltration of mononuclear cells, luminal dilatation of the glands exhibiting zymogen granule depletion, and an increased thickness of the whole mucosal layer.

KEY WORDS — *Bacteroides fragilis*, electron microscopy, rabbit, sinusitis, *Streptococcus pneumoniae*.

INTRODUCTION

An increased blood flow,[1] accompanied by acute inflammatory changes such as an infiltration of polymorphonuclear leukocytes and edema,[2] is a typical early finding in the course of experimental sinusitis in the rabbit. The immediate mucosal response is characterized also by focal lesions exhibiting epithelial disruption. Furthermore, areas with squamous or cuboidal cell metaplasia become evident within 1 week. An increase in periodic acid–Schiff (PAS)–positive secretory cells and regeneration of goblet cells in the epithelium within 2 weeks are also highly characteristic.[3] On exposure to an anaerobic *Bacteroides fragilis* infection, the sinus displays an even more pronounced inflammatory response, accompanied by new bone formation and zymogen granule depletion in the serous subepithelial glands.[4]

In the air passages, the mucociliary system is an important defense mechanism that is modified by numerous inflammatory mediators. Mediators may stimulate mucociliary activity at certain concentrations,[5-7] but at other concentrations and during in vivo conditions an impeded transport capacity has been demonstrated.[8] Various common respiratory conditions, eg, chronic sinusitis, are associated with an impaired mucociliary activity,[9] and the increase in retained mucus in chronic sinusitis is probably the result of both weakened ciliary activity and increased mucus production.[10] For the mucociliary clearance dysfunction seen in sinusitis, the rheologic properties of the mucus seem to be less important than the ciliary beating frequency and the periciliary fluid production.[11] A close correlation appears to exist between the degree of epithelial changes and the frequency of ciliary beating in chronic sinusitis.[12]

The present study was designed to describe the long-term structural changes that occur in the mucosa following acute experimental sinusitis. For this purpose, mucosal specimens from rabbits experimentally infected with *Streptococcus pneumoniae* and *B fragilis* were examined by light and electron microscopy 3 to 12 weeks after induction.

MATERIAL AND METHODS

A *B fragilis* infection was induced in the maxillary sinus of 18 rabbits by occlusion of the maxillary ostium with a polyacrylate plug and injection of 10^6 colony-forming units (CFU) of *B fragilis* strain NCTC 9343.[4] An acute pneumococcal infection was similarly induced in another 18 rabbits by ostial occlusion and injection of 10^8 CFU of *S pneumoniae* serotype 3 derived from a clinical isolate.[2] The animals were sacrificed 3, 4, 8, and 12 weeks after induction of the sinusitis. With the exception of 4 rabbits (described

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REPRINTS — Karl Magnus Westrin, MD, PhD, Dept of Otorhinolaryngology, Huddinge Hospital, S-141 86 Huddinge, Sweden.

below), fixation of the tissue was performed by intraarterial perfusion. The animals were sedated with an intramuscular injection of Hypnorm (fluanisone 10 mg plus fentanyl citrate 0.2 mg/mL; 0.25 to 0.40 mL/kg of body weight) and Stesolid (diazepam 5 mg/mL; 1.4 mg/kg of body weight), and after tracheotomy they were ventilated with an animal respirator (Harvard 665, Harvard Apparatus Ltd). After an intravenous injection of Pavulon (pancuronium bromide 2 mg/mL; 0.1 mg/kg of body weight) to prevent convulsions, the left heart ventricle was cannulated and 0.1 mol/L sodium cacodylate buffer with 0.1 mol/L sucrose was infused for 15 seconds to clear the blood vessels. Fixation was initiated by perfusion of a solution containing 3% glutaraldehyde in the same cacodylate buffer, at pH 7.4, maintaining a hydrostatic pressure of 100 cm H_2O for a period of 10 minutes. The sinuses were immersed in the same fixative until dissected under a microscope.

Strips of the mucosa were removed from the same site in all animals, ie, the lateral wall in the anterior part of the middle compartment of the maxillary sinus. For reference purposes, normal mucosal specimens from 4 sinuses of healthy animals were obtained similarly. Furthermore, mucosae from the contralateral, noninfected sinus of 6 rabbits with sinusitis also served as controls. After a rinse in cacodylate buffer, the specimens were postfixed in 1% osmium tetroxide in the same buffer, dehydrated in a graded series of ethanol, and embedded in Epon 812.

One-micron sections were cut and stained with 1% toluidine blue. The slides were examined in a projection light microscope equipped with a square lattice, for intersection point counting morphometry of the relative volume occupied by different cellular populations in the epithelial lining as described by Weibel.[13]

Ultrathin sections about 70 nm thick were cut with a diamond knife in a Ultratome III (LKB-produkter, Bromma, Sweden), mounted on 100-mesh copper grids covered with a Formvar (polyvinyl) film, and contrasted with uranyl acetate and lead citrate in an automatic contraster (Ultrastainer, LKB-produkter). The preparations were examined in a JEM 1200 EX transmission electron microscope (JEOL Ltd, Tokyo, Japan). The size of cells and organelles, as well as epithelial thickness, were determined in micrographs by comparing the images with an intrinsic scale of the electron microscope.

Two rabbits from each group, surviving 8 and 12 weeks, were painlessly sacrificed with an intravenous overdose (300 mg) of Mebumal Vet. The mucosa was prepared for light microscopy with hematoxylin and eosin, van Gieson, and Alcian blue–PAS (pH 1.0 and 2.5) stains after immersion fixation in phosphate-buffered 4% formalin, decalcification in 24% ethylenediaminetetraacetic acid, paraffin embedding, and sectioning of the whole-mounted nose complex at 5-μm thickness.

When not stated otherwise, frequency and dimension values are given as median plus or minus quartiles of range.

RESULTS

Normal Mucosa. The mucosa of the normal rabbit sinus is lined by a pseudostratified columnar epithelium containing ciliated cells, nonciliated dark cells, and basal cells (Fig 1A). The height of the normal epithelium is 17 ± 2.5 μm. The cilia are of uniform length (range 7.5 to 8.5 μm), and 0.2 μm in diameter (Fig 1B). The dark cells, occupying 10% ± 2.4% of the epithelial volume, may contain granules of differing diameter and electron density. At least some of these granules, usually located in the apical portion of the cytoplasm, stain magenta with PAS. There are no or only few "classic" goblet cells in the normal epithelium. The subepithelial glands are typical serous glands, with pyramid-shaped secretory cells. They contain abundant electron-dense zymogen granules, which stain magenta with PAS. The collagenous connective tissue surrounding the glands is normally rather delicate (Fig 1C).

Pneumococcal Sinusitis. Some 3 to 4 weeks after induction of an *S pneumoniae* infection, the epithelial layer was generally thicker (22 ± 3.3 μm) than in normal controls (Fig 2A). Goblet cells could be observed scattered between ciliated cells (Fig 2B), their distribution varying locally (range 0% to 12%). The relative volume of the epithelium occupied by basal cells was not markedly affected. Furthermore, the relative volume of dark cells (6.2% ± 2.9%) was slightly reduced. In certain specimens at the 3- and 4-week intervals, there appeared mucosal areas displaying loss of ciliated epithelium replaced by nonciliated cells, as well as slight focal infiltration of plasma cells, lymphocytes, and granulocytes.

Presence of goblet cells and focal fibrosis of the lamina propria was noted in 3 of 6 animals even after 8 and 12 weeks (Fig 2C). The goblet cells appeared to be located chiefly in areas adjacent to subepithelial mucosal lymphoid aggregates. The intercellular space in the basal portion of the epithelium appeared to be reduced, compared with normal animals (Figs 1B and 2A,B). An impression of a correlation between increasing degree of inflammation-edema and reduced intercellular space was gained from the present material.

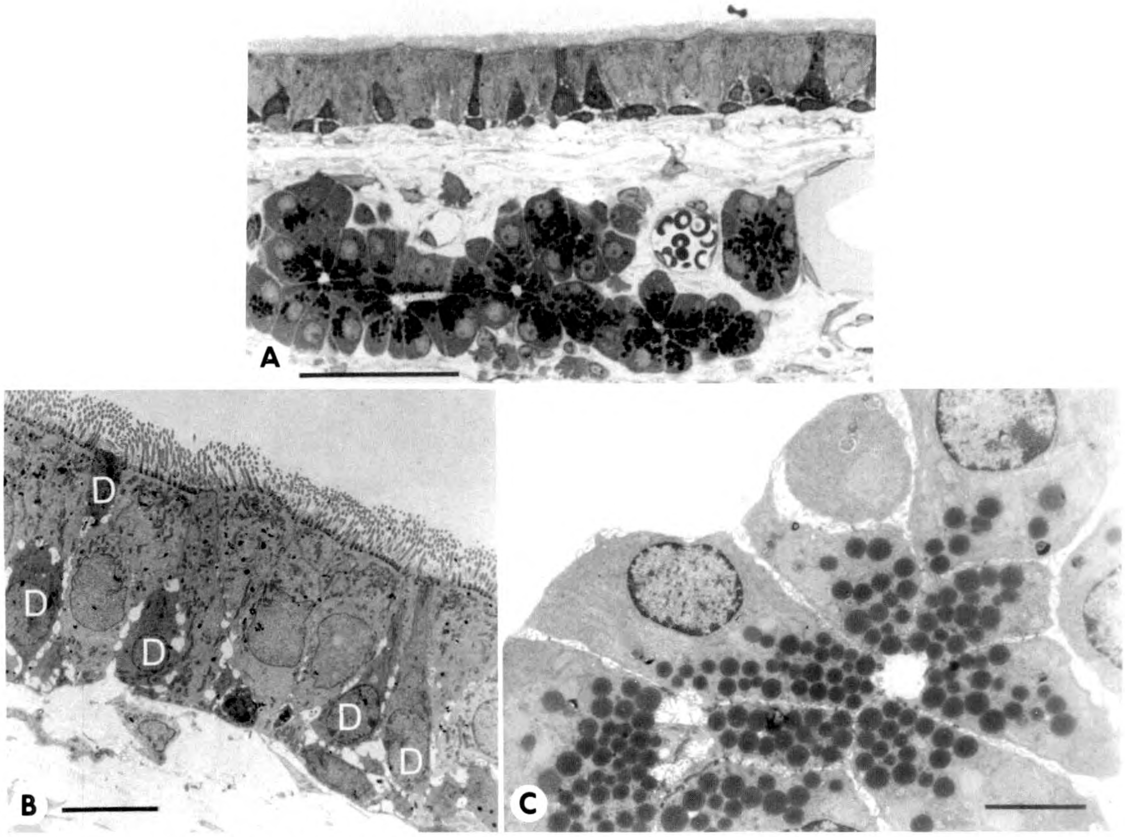

Fig 1. Normal maxillary sinus mucosa of rabbit. A) Light micrograph (toluidine blue, x400). Ciliated cylindrical cells of fairly uniform shape constitute bulk of epithelial cell layer. Dark cells, sometimes with granules, and basal cells can also be recognized. Subepithelial serous glands contain numerous dark granules. Bar — 50 μm. B) Transmission electron micrograph of same specimen as in A (uranyl acetate and lead citrate, x1,250). Abundant mitochondria are located below cilia. Dark cells (D) contain numerous microvilli but no cilia. Bar — 10 μm. C) Electron micrograph of serous glandular cells with numerous dense spheroidal granules in normal sinus tissue (uranyl acetate and lead citrate, x3,200). Microvilli extend into lumen. Moderate number of interdigitating folds can be seen between pyramidal cells. Note thin collagen bundles surrounding gland, sectioned transversely. Bar — 4 μm.

Bacteroides *Sinusitis*. The mucosal changes seen 3 and 4 weeks following sinusitis induced with *B fragilis* were much more pronounced than those observed in pneumococcal sinusitis. The thickness of the epithelial layer was 25 ± 7.5 μm, reaching in certain areas a maximum of 43 μm. An increased number of nonciliated cells displaying only short microvilli on the luminal surface was present at this time interval. These cells were often dome-shaped and protruded slightly into the sinus lumen. Their microvilli were occasionally separated by blebs of the apical cell membrane (Fig 3A). The microvilli were often deformed, and the characteristic accumulation of mitochondria in the apical part of the cytoplasm seen in ciliated cells was absent. The relative volume of the basal cell population was similar to that of the controls. After 3 to 4 weeks, large numbers of goblet cells were present in *Bacteroides* sinusitis (range 0% to 32%). However, goblet cells were sparse in areas characterized by marked inflammatory changes and areas in which the ciliated epithelium was replaced by nonciliated cells.

There was dense infiltration by plasma cells and by lymphocytes and granulocytes to the mucosa of *B fragilis*–infected specimens (Fig 3B). In subepithelial glands there was often a reduced amount of secretory granules, showing considerable variation in size, with a predominance of small granules. The glandular lumina were dilated and contained some flocculent and membranous material (Fig 3B,C). Focally, a heavy collagen deposition in the lamina propria was encountered.

The inflammatory changes in the *Bacteroides* group observed after 8 to 12 weeks were less pronounced than after 3 and 4 weeks, yet more severe than the long-term changes observed in the pneumococcal group. Purulent secretions and mucosal edema were present in most of the 8- and 12-week rabbits. Granule depletion of the subepithelial serous glands (Fig 3C), round cell infiltration (mainly lymphocytes and plasma cells), and focal fibrosis were particularly

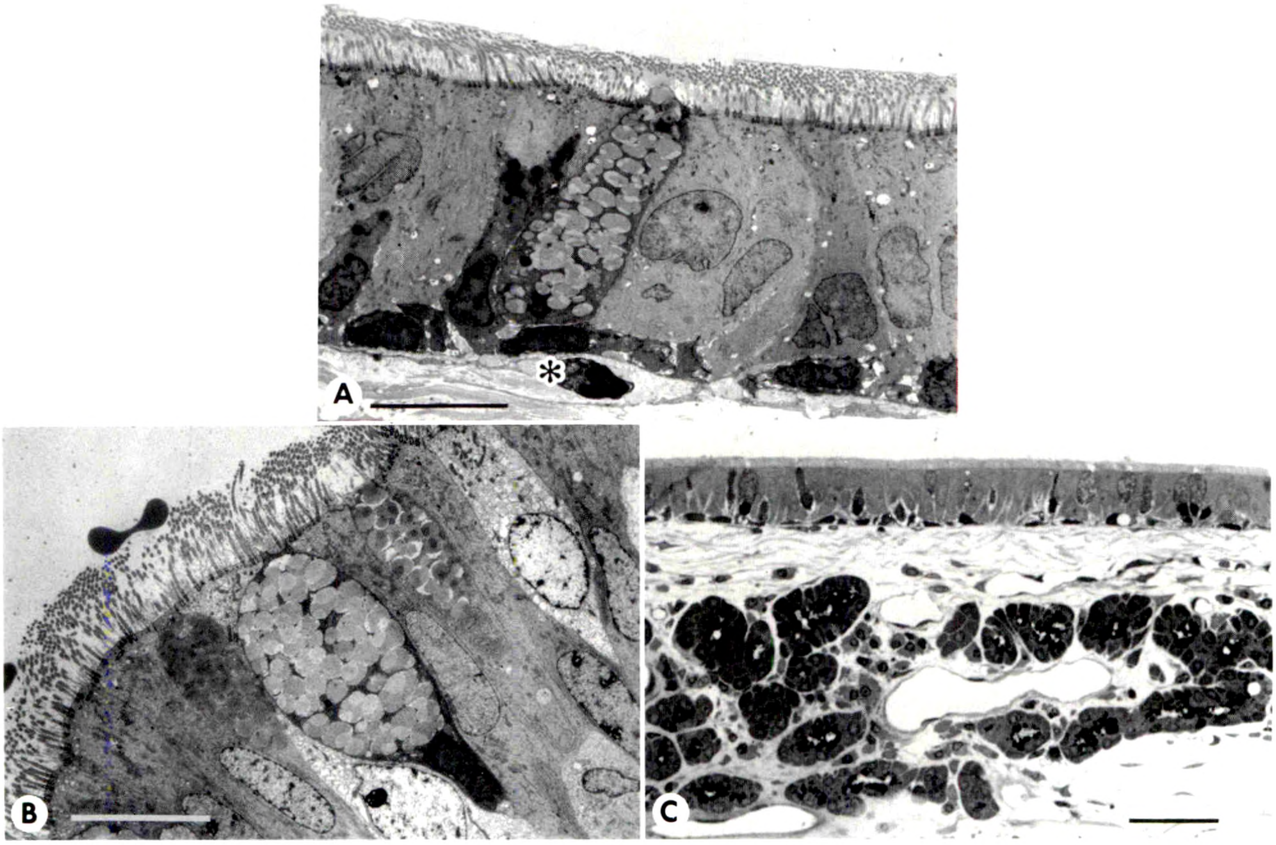

Fig 2. Rabbit maxillary sinus mucosa infected with pneumococcal sinusitis. A) Electron micrograph of epithelium after 3 weeks of sinusitis (uranyl acetate and lead citrate, x1,750). In center is goblet cell with confluent mucous granules. There appear to be fewer apically clustered mitochondria in ciliated cells, and intercellular spaces appear to be narrowed. Asterisk — granulocyte, bar — 10 µm. B) Electron microscopic preparation of sinus mucosa after 4 weeks of sinusitis (uranyl acetate and lead citrate, x1,750). Appearance of secretory cells is varied, containing granules ranging from mainly dark to pale with dark core. There is typical goblet cell with mainly pale confluent granules in center. To right, ciliated cell can be seen with highly vacuolized cytoplasm and confluent cilia (signs of cellular degeneration). Bar — 10 µm. C) Light micrograph of sinus mucosa 12 weeks after induction of sinusitis (toluidine blue, x230). Epithelial layer appears to be almost normal. However, goblet cells are intermingled with ciliated cylindric cells, and relative number of dark cells is markedly reduced, compared with normal (seen in Fig 1A). Number of granules in subepithelial glands appears to be reduced. Bar — 50 µm.

characteristic. The cilia had often regained a fairly normal appearance, even in sinuses in which other inflammatory changes persisted (Fig 3D). However, localized loss of the ciliary lining was occasionally noted. The increased number of epithelial secretory cells persisted, and the volume of goblet cells varied among different biopsy specimens (range 0% to 17%). The finding of goblet cells clustering mainly in the vicinity of lymphoreticular aggregates, characteristic of the later stages in the pneumococcal group, was not a prominent feature in the *Bacteroides* group.

DISCUSSION

In the present study, sinusitis could be induced in occluded rabbit maxillary sinuses by *S pneumoniae* and *B fragilis*. The two microorganisms provoked different long-term cell responses within the rabbit mucosa. Spontaneous sinusitis can occur in rabbits with respiratory tract infection.[14] *Pasteurella multocida*, in particular, has been reported as a major airway pathogen, carried by more than 90% of one examined rabbit population in the United States.[15] However, the animals used in the present study were all *Pasteurella*-free by culture, as was the stock from our laboratory animal purveyor, according to repeated veterinary controls.

Normal human sinus mucosa exhibits scattered subepithelial tubuloacinar glands, but no true glandular layer,[10] whereas goblet cells are fairly evenly distributed throughout the epithelial lining at a concentration of approximately 10,000 cells per square millimeter.[16] An increase in goblet cell frequency is often found during airway infections (see, for example, the report by Tos[16]). In chronic maxillary sinusitis, it has been reported that hyperplasia occurs in the tubular subepithelial glands, whereas the number of goblet cells decreases.[10] In contrast to the human maxillary sinus, the healthy rabbit mucosa lacks mucous goblet cells, but its lamina propria has a fairly evenly distributed layer of serous glands. The muco-

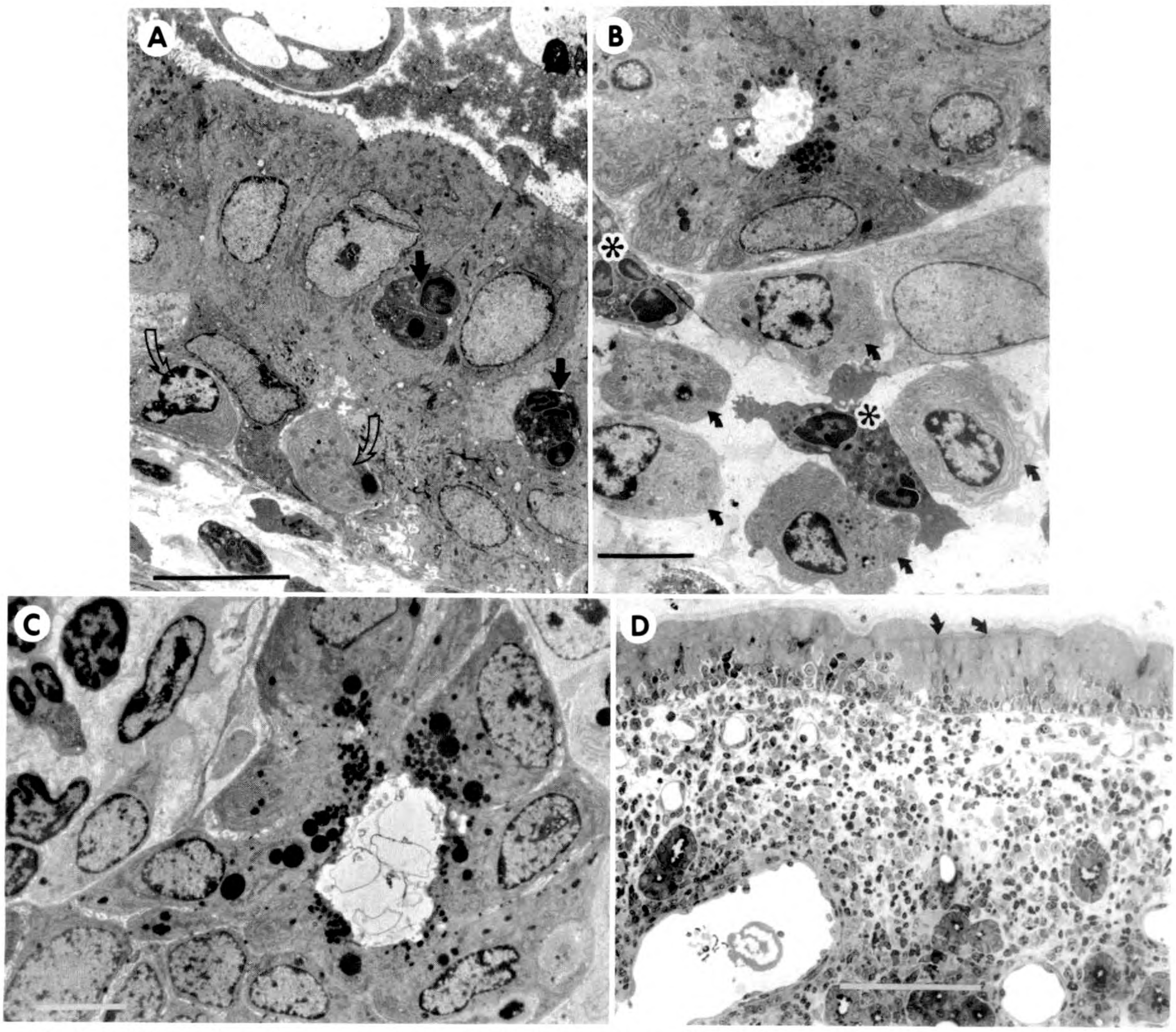

Fig 3. Rabbit maxillary sinus mucosa infected with *Bacteroides fragilis*. A) Electron micrograph of intensely inflamed mucosa, infected for 3 weeks (uranyl acetate and lead citrate, x1,750). At top left, some irregular cilia can be observed; other epithelial cells are covered with short, abnormal microvilli. At right, cytoplasmic bleb bulges into sinus cavity. Slightly increased content of heterochromatin is seen in nuclei of epithelial cells (sign of increased protein synthesis). Amorphous secretion fills lumen. Granulocytes (filled arrows) and plasma cells (open arrows) are seen penetrating epithelium. Bar — 10 μm. B) Electron micrograph of serous gland in 3-weeks–infected specimen with abnormally small zymogen granules (uranyl acetate and lead citrate, x2,500). Plasma cells (arrows) and polymorphonuclear granulocytes (asterisks) have infiltrated mucosal lamina propria. Flocculent and membranous material is deposited in lumen. Bar — 5 μm. C) Electron micrograph of serous gland after 8 weeks of *B fragilis* infection (uranyl acetate and lead citrate, x2,350). Number as well as size of electron-dense granules is still reduced. Lumen is dilated, and there are no normal microvilli projecting into it. Intensified intercellular activity is indicated by increasingly interdigitating folds at cellular junctions. Bar — 5 μm. D) Light micrograph of mucosa after 8 weeks of *B fragilis* sinusitis (toluidine blue, x190). Epithelial layer is thicker than normal, with activated basal cell population. Nuclei of cylindric cells occur at different levels. Occasional goblet cells can be seen (arrows). Profuse infiltration by inflammatory cells, particularly round cells, in both lamina propria and epithelium can be seen. In spite of intense inflammation, ciliary carpet appears to be almost normal. Bar — 100 μm.

sal samples described in this paper were collected from the same location in all sinuses, namely the lateral wall of the middle sinus compartment. This origin provides a mucosa with a representative histopathologic reaction, while the risk of artifacts from surgical trauma is minimal.

It could be surmised that the reduced number of granules observed in the subepithelial glandular cells, particularly in the *B fragilis* group, might be caused by an impaired synthesis of zymogen granules caused by energy depletion and low oxygen tension in the inflamed tissue. However, increased secretory rate might also account for the granule depletion. It has been demonstrated that the activity of the energy-requiring enzyme regulating sodium and potassium transport increases significantly in the rabbit tubuloacinar glands during purulent sinusitis, indicating an increased secretory rate.[17] Thus, the infection not only enhances the secretory capacity of the sinus mucosa by inducing goblet cell formation, but also has a stimulatory effect on the serous glands. In an attempt to compare this increase in secretion to what has been described in human studies (by, for example, Tos and Mogensen,[10] Majima et al,[11] and Ohashi and Nakai[12]), it would appear that the adaptation of the secretory capacity of the infected mucosa in each species is limited by its genetically determined transformation potential.

In the present study it was noted that the relative volume occupied by basal cells was not altered by the inflammatory condition, in spite of the increased thickness of the whole epithelium. This finding is considered to be the result of hyperplasia of basal cells, combined with hypertrophy and/or hyperplasia of the cylindric cell moiety. The basal cell layer is assumed to be activated by the healing process.[18] A layer of intact basal cells on the basal membrane can promote complete regeneration of the epithelium within 5 days, as seen in the nasal cavity.[19] In the rabbit airway, basal cells can generate secretory cells with various morphological characteristics, as well as ciliated cells, and can thus be regarded as epithelial stem cells.[18]

In the maxillary sinus mucosa, pluripotent basal cells and "resting" dark cells may transform into goblet cells. A transformation induced by airway infection, from serous to mucous epithelial cells, has been demonstrated in the pathogen-free rat,[20] and a similar transformation of secretory cells could explain the appearance of modified epithelial secretion products during the course of experimental sinusitis.[3] Furthermore, in the piglet, experimental airway infection can cause gland hypertrophy, increasing the number and size of the secretory cells,[21] and causing an increase in acid glycoprotein compared to neutral. As can be seen in this model, the presence of goblet cells in experimental sinusitis indicates a state of relative recovery, whereas the most severely damaged mucosae lack goblet cells. In particular, goblet cells with a high concentration of sulfated mucin seem to signify secretory maturation, since they were found to be more predominant where the mucosal defense had taken control and the infecting microorganism was eradicated.

The great number of plasma cells seen in *Bacteroides*-infected mucosa, characteristic of chronic inflammation,[22] implies a content of potent "polyclonal B-cell activators" in *B fragilis*. Not only is a prevalence of a specific antigen and nonspecific activators necessary at the local site in order to induce plasmocytic invasion — so, too, is a recent antigen-induced activation of antigen-specific lymphocytes and a local environment propitious for chronic infection.[22]

The formation of specific antibodies to the infecting microorganisms and the early signs of mucosal restoration in these sinusitis models have been reported previously.[3,4,23,24] The induced infections elicit a vigorous serum antibody response, and the specific antibody activity is still vigorous in serum even 12 weeks after the onset.[23,24] In addition to the regeneration of immunocompetent cells in the lamina propria of the sinus, an increase in collagen was also noted in this area. It has been reported that epithelial necrosis and delayed repair promote fibroblast growth,[25] and the collagen deposition process is probably initiated at an early stage, when epithelial desquamation is severe.

To conclude: this model infection exposes a previously sterile or uncolonized mucosa to a single isolated bacterial challenge. In *Bacteroides* sinusitis, the histology reflects a prolonged anaerobic microbial challenge,[4] whereas the histologic picture in pneumococcal sinusitis is the result of a brief, self-limiting infection, as has been documented previously.[3]

Although some morphological variation may be expected within each group, the degree of mucosal damage was generally correlated to the virulence of the infectious agent, as well as to the period during which it was able to exert its toxic effects on the tissues. The induced ostial occlusion in this model hinders the mucociliary clearance of the sinus cavity, and this probably helped to prolong the course of the inflammatory condition. Moreover, colonization by microorganisms from the rabbit nasopharyngeal flora, which occurs in pneumococcal sinusitis,[3] may have helped to prolong some of the histologic changes. As has been reported in the middle ear,[26] a second line of mucosal defense could be demonstrated. Except for the increased epithelial thickness and an increased glandular and epithelial secretory activity, local mobilization of immunocytic cells was induced.

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CRITICAL CARE MEDICINE: A COMPREHENSIVE REVIEW AND UPDATE

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IMAGING CASE STUDY OF THE MONTH

GIANT CONGENITAL CHOLESTEATOMA

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FERNANDO MARTIN, MD
ANTONIO SODA, MD
MEXICO CITY, MEXICO

CASE REPORT

A 19-year-old woman presented to the outpatient department with a long-standing left-sided hearing loss (first noticed at the age of 8), and occasional dizziness and unsteadiness for approximately 2 years. She had been seen by an otolaryngologist 1 year previously and was told that her hearing loss was probably viral and that there was no need for further treatment.

On examination, the eardrum was found to be normal and intact. The Weber test lateralized to the right, and the Rinne test was normal on the right and absent on the left. An audiogram showed anacusis on the left. A Schuller's projection was requested (Fig 1), followed by computed tomography (CT; Fig 2) and magnetic resonance imaging (MRI; Fig 3).

RADIOLOGIC FINDINGS

The Schuller's view (Fig 1) shows extensive erosion of the temporal bone and loss of mastoid air cells on the left side. The right side is normal.

Computed tomography demonstrates almost complete destruction of the mastoid and petrous ridge with conservation of some of the middle ear structures and the external auditory meatus bone. There is erosion into the middle fossa and into the posterior fossa. The lesion does not enhance (Fig 2).

An MRI scan demonstrates a lesion, especially in the T2 mode, of high lipid content in the area of the mastoid bone and petrous apex, extending into the posterior fossa and down to the foramen magnum with considerable compression of the cerebellum (Fig 3).

SURGICAL CORRELATION

On June 26, 1989, the patient underwent an extended mastoidectomy. The cholesteatoma encountered was found to expose the posterior fossa dura for a distance of approximately 10 cm, with medial displacement of the lateral sinus and cerebellum down to the foramen magnum. There was complete destruction of the temporal bone and inner ear structures. The facial nerve was found in the normal location, partly dehiscent in its descending portion.

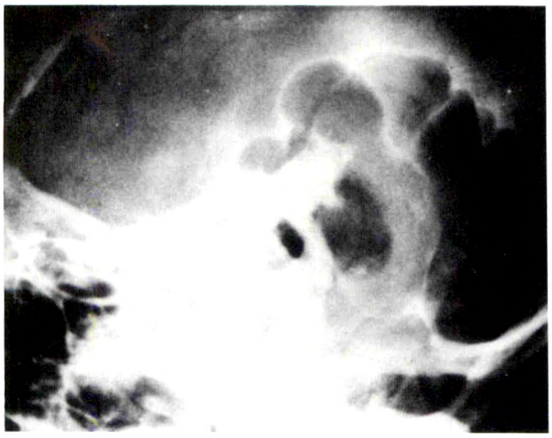

Fig 1. Schuller's projection showing extensive erosion of left temporal bone and loss of mastoid air cells.

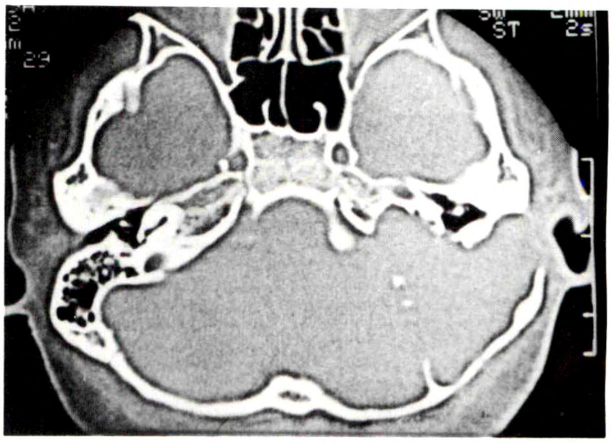

Fig 2. Computed tomogram demonstrating almost complete destruction of mastoid bone and petrous apex with erosion into posterior and middle fossae.

From the Departments of Otolaryngology (Borgstein, Soda) and Radiology (Martin), National Institute of Respiratory Diseases, Mexico City, Mexico.

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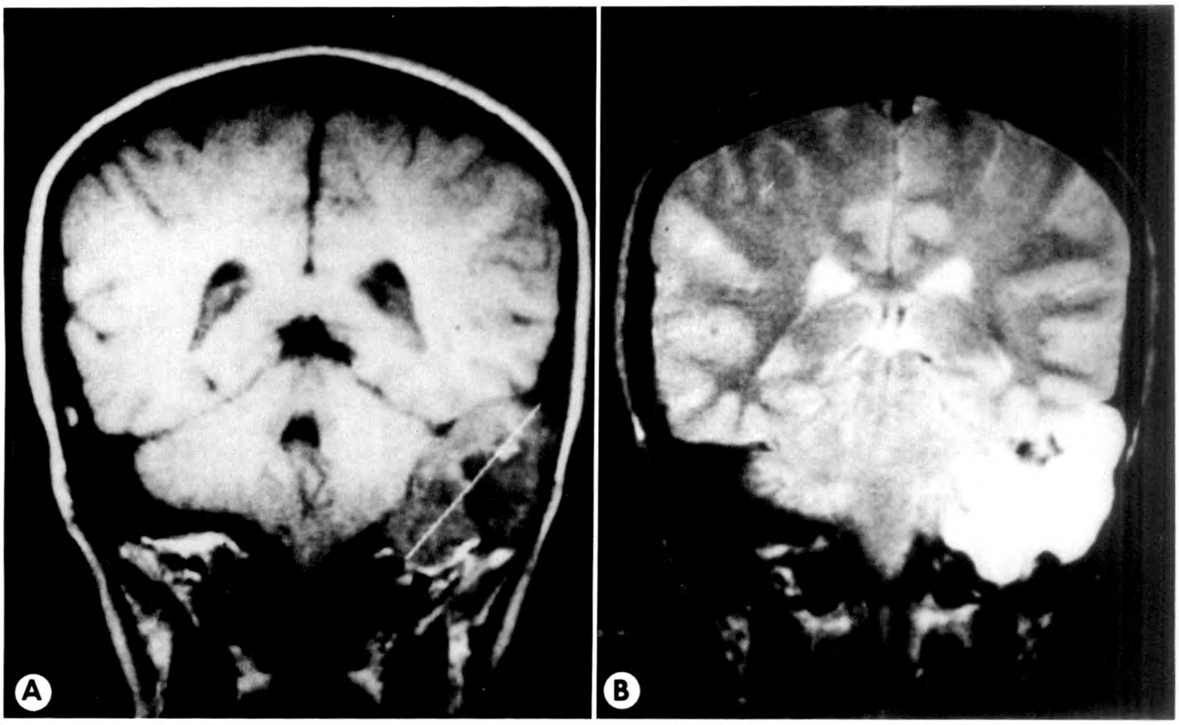

Fig 3. Magnetic resonance images showing A) large destructive lesion of B) high lipid content replacing petrous apex and extending in posterior fossa down to foramen magnum, with compression of cerebellum.

The cholesteatoma was removed completely, revealing considerable fibrosis of the dura. A small cerebrospinal fluid leak was sutured and sealed. The patient recovered completely from a slight facial weakness within 2 weeks.

DISCUSSION

The long history and absence of cholesteatoma in the middle ear and aditus indicate a probable congenital origin of this cholesteatoma.[1-3] The destruction of the inner ear went entirely unnoticed by the family, until at the age of 8 years the patient first noticed a hearing loss on the left side that did not appear to be progressive. In spite of complete destruction of the labyrinth, there was no history of vertigo or dizziness until occasional episodes of unsteadiness that started 2 years prior to surgery; they were probably due to compression of the cerebellum and brain stem. The postoperative recovery was unremarkable, and a repeat CT scan taken 2 years after surgery revealed no evidence of recurrent cholesteatoma.

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FUNCTIONAL SINUS TECHNIQUES

An international course on functional sinus techniques will be held in Graz, Austria, September 2-4, 1993. It will be conducted in English. For further information, contact Prof Dr Heinz Stammberger, University ENT-Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; telephone 43-316-385-3448; fax 43-316-385-3425.

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PATHOLOGY CONSULTATION

PAPILLARY NEOPLASMS (HEFFNER'S TUMORS) OF THE ENDOLYMPHATIC SAC

JOHN G. BATSAKIS, MD ADEL K. EL-NAGGAR, MD, PHD

HOUSTON, TEXAS

The sources of adenomatous neoplasms in the temporal bones are usually metastases or direct extensions from extratemporal lesions, or primary from the middle ear cleft. In 1989, Heffner added the endolymphatic sac's epithelium as another possible generative origin. In contrast to the adenomatous tumors of the middle ear or mastoid, the papillary cystic neoplasms of the endolymphatic sac are large and locally aggressive and often involve the middle and posterior cranial fossae and bone. These biologically low-grade adenocarcinomas have not been shown to be able to metastasize.

Just a little less than a quarter of a century ago Shambaugh et al[1] wrote that the endolymphatic sac and its duct were almost forgotten by clinicians and virtually ignored by pathologists. This deficiency has certainly been rectified, but our knowledge of these structures is still incomplete. Until the classic paper by Heffner,[2] neoplasms of the sac or duct were hardly acknowledged. In this report we summarize the contemporary information relating to these rare lesions.

DEVELOPMENT AND ANATOMY OF ENDOLYMPHATIC SAC

The inner ear is divided into 3 parts: 2 sensory (the cochlea and the vestibular system), and 1 nonsensory part of the membranous labyrinth (the endolymphatic duct and sac). The sac and duct are embryologically derived from neuroectodermal stem cells of the otocyst and differentiate morphologically and functionally throughout gestation.[3-7] The sac continues to undergo considerable change in the late fetal and postnatal periods.[4,5]

Most of the internal ear becomes encased in the bony otic capsule shortly after midterm.[6] The endolymphatic sac, which is the terminal saccular enlargement of the endolymphatic duct, lies between the inner and outer layers of the dura and the posterior surface of the petrous portion of the temporal bone, where it can expand with the growing dura.[6] At all ages the sac is leaflike, with its proximal part connecting to the endolymphatic duct much as a leaf is attached to its stem.[6] Three regions of the sac are commonly recognized: the proximal or rugose region, the sac proper, and the distal projection. In adults, the distal projection is essentially absent, as it is absorbed by the sac proper.[6] Especially noteworthy is the observation that the medial margin of the endolymphatic sac is always near the sigmoid sinus and the jugular bulb.[6] One of the reasons the endolymphatic sac's microanatomy has been elusive is that during the preparation of petrous-temporal bones for histologic sections the dura mater on the posterior surface is liable to be torn off, thus destroying the sac, which is lodged in it.

ENDOLYMPHATIC SAC EPITHELIUM

The premise that the endolymphatic sac is a dynamic organ, capable of modifying its structure according to requirements (eg, autoregulation of the inner ear's fluid and ion balance and maintenance of endolymphatic pressure), is supported by the observed variability in size, shape, and polymorphism of its epithelial cells and by electron optic findings, as well as immunocytochemical reactions of the cells.[3,8-13] It is also evident the endolymphatic sac is immunocompetent and plays an active role in the immunodefense of the inner ear.[3] A secretory function of the epithelium of the sac is evinced by immunoreactivity, with antibodies directed against cytokeratins 18 and 19 and epithelial membrane antigen.[3] Secretory immunoglobulin A is also present in the epithelium.[3] Resorptive, as well as secretory, properties are suggested by epithelial cell coexpression of vimentin and cytokeratin intermediate filaments.[3] Strong immunoreactivity with antibodies specific for S-100 protein antigen in epithelial cells speaks for a high degree of metabolic activity.[3] Finally, a paracrine activity of the endolymphatic sac is implied, in that a few of its cells demonstrate neuroendocrine granules and an immunoreactivity for neuron-specific eno-

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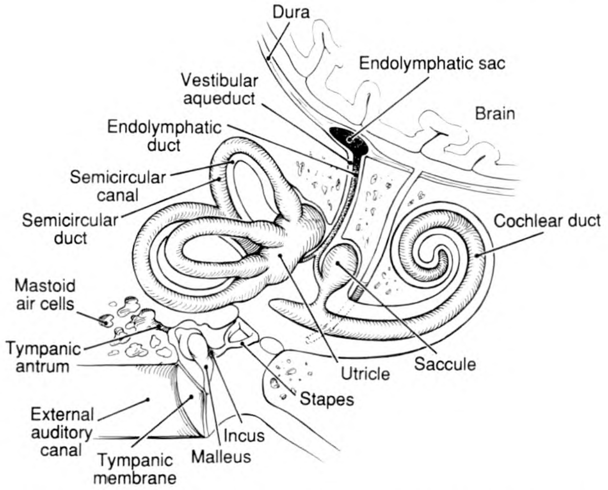

Fig 1. Schematic depiction of endolymphatic sac and its relationship to dura, brain, and inner and middle ear.

lase, chromogranin, and somatostatin.[3,9,11]

ENDOLYMPHATIC SAC PAPILLARY TUMORS

Benecke et al,[14] extending observations by Batsakis[15] and Gaffey et al,[16] divided adenomatous tumor involving the middle ear and mastoid into 2 clinico-pathologic groups: mixed and papillary. As used by Benecke et al,[14] "mixed" refers to the more common of the 2 patterns and includes tumors with an acinar, solid, trabecular, cribriform, or carcinoidlike architecture. Of the examples of this type reported by Benecke et al,[14] the tumor was 1) always confined to the middle ear and mastoid, 2) commonly misdiagnosed as chronic otitis media, and 3) rarely demonstrated an involvement of the otic capsule or the facial nerve. Papillary tumors, on the other hand, always had extensions to the petrous apex, frequently involved the middle and/or posterior cranial fossae, and usually involved the facial nerve.

The papillary tumors had a female dominance; the mixed-type adenomatous tumors, a male preference. The age of patients at time of clinical presentation was not dissimilar between the two, with the aggregate having an age range of 19 to 57 years.

Imaging studies of the mixed adenomatous tumors showed soft tissue densities in the middle ear and mastoid, but without bone destruction or extensions into the petrous apex or intracranially. The aggressive papillary tumors involved temporal bones and manifested intracranial extension.

The papillary adenomatous tumors described by Benecke et al[14] are related to, if not the same as, those reviewed by Gaffey et al,[16] but it was in Heffner's[2] superb analysis (light- and electron-optic and immunochemical) of 20 papillocystic temporal bone tumors that the endolymphatic sac, especially its rugose portion, rather than the middle ear was postulated as the generative source for the tumors.

Most of the reported examples of the papillary tumors have been advanced lesions, hence obscuring the site of origin. Radiologically, however, the lesions are often centered between the sigmoid sinus and the internal auditory canal along the posterior petrosal plate, in the region of the vestibular aqueduct.[2] Other findings are erosion of bone toward the vestibule of the labyrinth (related, perhaps, to spread along the endolymphatic duct) and involvement of the internal auditory canal, jugular bulb, and medial mastoid. There is often invasion of the posterior fossa's dura. This may be a result of extensions from the extraosseous part of the endolymphatic sac, which protrudes in the retrolabyrinthine, presigmoid area.

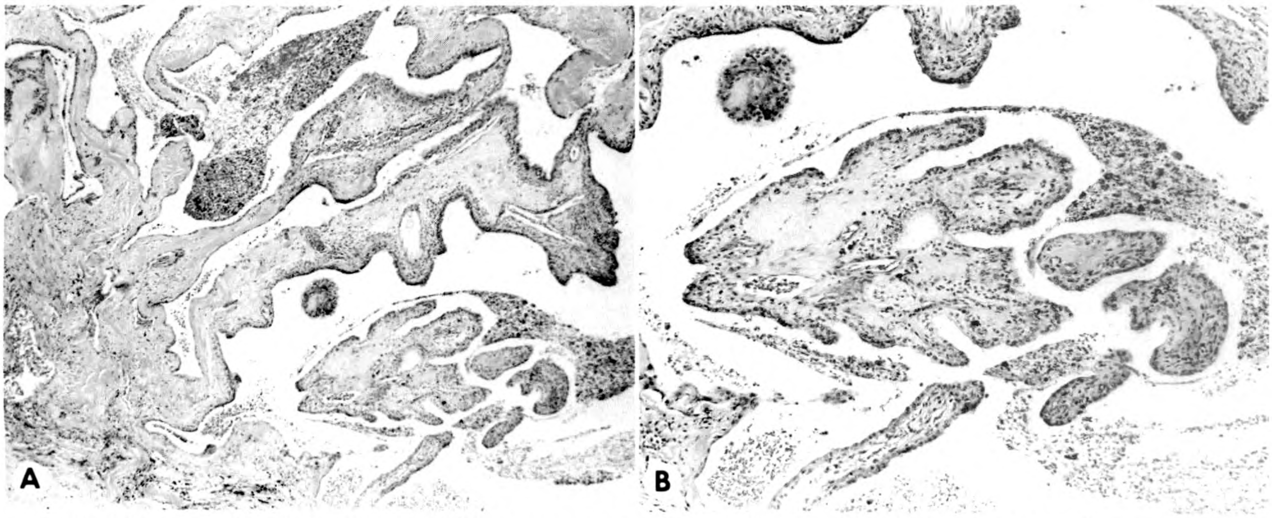

Fig 2. A) Small papillary lesion (lower right) in lumen of what appears to be distorted and fibrotic endolymphatic sac (H & E, original x40). B) Higher magnification of papillary lesion in A (H & E, original x110).

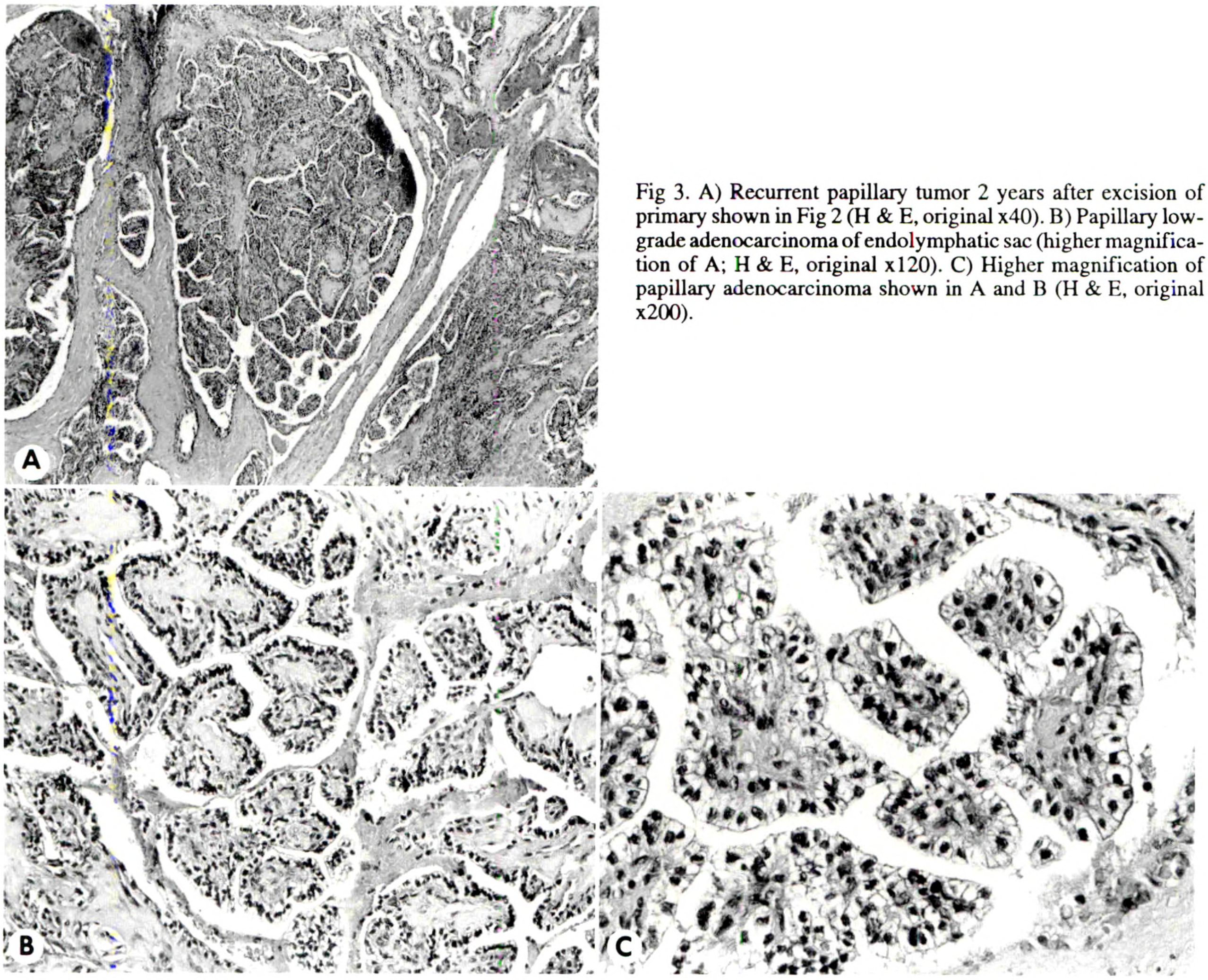

Fig 3. A) Recurrent papillary tumor 2 years after excision of primary shown in Fig 2 (H & E, original x40). B) Papillary low-grade adenocarcinoma of endolymphatic sac (higher magnification of A; H & E, original x120). C) Higher magnification of papillary adenocarcinoma shown in A and B (H & E, original x200).

These radiologic findings are in accord with the tumor's growth as postulated by Poe et al.[17] These authors suggest the tumors begin silently within the endolymphatic sac and grow into the dura of the posterior fossae and the endolymphatic duct. Gaining access to the vestibule, the tumors erode the semicircular canals and expand the mastoid process inferior to the posterior canal and along the retrofacial cells, where the facial nerve can be involved. Continued growth can destroy the otic capsule, promote invasion of the middle ear, and eventually involve the middle and posterior fossae and the base of the skull (Fig 1).

Loss of hearing and vertigo are the most common symptoms in patients with the papillary tumors. Progression of the tumor leads to facial nerve weakness and a vascular mass in the middle ear. It is recommended that patients with an unexplained, asymptomatic sensorineural hearing loss and vertigo should have magnetic resonance imaging as the diagnostic evaluation of choice.[17]

Patients with von Hippel–Lindau disease appear to be especially predisposed to developing papillary tumors of the temporal bone.[17] In some patients with the autosomal dominant disorder the papillary tumors have been bilateral.

Despite their often large size and locally destructive behavior, the papillary tumors have not been shown to metastasize. The primary treatment is complete surgical excision using combined neurosurgical and transtemporal skull base techniques.[17]

The papillary tumors with an indisputable origin from the endolymphatic sac reported by Hassard et al[18] and Poe et al,[17] Heffner's[2] demonstration of the striking similarities between the papillary adenomatous tumors and the epithelium of the endolymphatic sac, and the clinicopathologic constancy of the tumors are rather convincing evidence of a non–middle ear origin.

Figure 2 shows the primary and Fig 3 the recurrent lesion of a temporal bone and represent evolution of an endolymphatic sac papillary tumor. Figure 3C is identical to Heffner's Figure 13 illustrating a known endolymphatic sac tumor.[2]

The general characteristics of the tumors are described by Heffner.[2] Papillary-cystic in growth, the tumors are variable in their cellularity, not only between tumors, but also within a given tumor. Many have areas of hypocellular fibrosis, old and recent hemorrhage, cholesterol granuloma areas, and related reactive changes. Where glandular lumens or larger cysts are present, they often contain a periodic acid–Schiff–stained colloidal fluid, and that, along with the papillae, can simulate a thyroid neoplasm. The cells lining the papillae are rarely multilayered and are usually distributed in either a single or double row. An optical clarity of the cytoplasm, in some cases containing glycogen, is a rather distinguishing feature, in our experience. Mucin may be demonstrated in isolated cells and in the cystic fluid. Attenuation of the epithelial cells is often seen, but prominent pleomorphism and mitotic activity are never a feature. When flattening of the epithelium is marked, the demarcation from underlying stroma is blurred.

While clearly adenomatous, the papillary lesions of the endolymphatic sac lack the generally accepted histologic features of malignancy. On the other hand, their intimate relationships to the dura and cranial fossae, expansile growth, and involvement of surrounding bone not only facilitate a locally aggressive behavior, but serve to characterize the lesions as histologically low-grade malignancies, ie, papillary adenocarcinomas.

From a surgical pathologist's view, these papillary neoplasms are distinctive enough to allow separation from the adenomatous tumors of the middle ear cleft. It should be remembered, however, that there are papillary neoplasms of the middle ear, albeit rare, that can also show a locally aggressive behavior.[15]

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BOOK REVIEWS

Rigid Fixation of the Craniomaxillofacial Skeleton

Michael J. Yaremchuk, Joseph S. Gruss, and Paul N. Manson, editors. Hard cover, illustrated, indexed, 696 pages, 1992. Butterworth-Heinemann, Stoneham, Mass, $225.

The revolution in surgery of the facial bones began in the seventh decade of this century. It was introduced independently in 2 centers in Europe, where both laboratory and clinical investigations were performed that demonstrated the efficacy of plate and screw fixation of mandibular fractures. Various implants and marked improvements in techniques for anesthesia were also introduced during this time. As a result, early bone healing could be accomplished through mobilization of facial fractures by open reduction.

This volume is intended to provide minute details of surgeries for trauma, orthognathic surgery, congenital craniofacial deformities, and tumors. These procedures are compiled in 3 of the 5 sections of the book. There are 56 authors, only 2 of whom are otolaryngologists.

Section I addresses basic concepts of rigid fixation, such as bone healing; compression osteosynthesis; techniques of lag screw osteosynthesis; a discussion of implant materials, including resorbable biomaterials; and a review of the effects of mandibular immobilization on the mastication muscles. The second section discusses the various existing systems for rigid fixation of the craniomaxillofacial skeleton.

The main attribute of this volume is the clarity of the written text. Each chapter follows a similar format and can be easily read — especially notable for such a massive book. There is liberal use of simple and functional photographs and radiographic illustrations.

Incidentally, in the development of this specialty, microplates emerged later than 1970. They are used in fixation of orbital advancement and in reshaping the cranial vault, since they can maintain gaps between bone edges. (Wires require interpositional bone grafts.) Chapter 22 gives an excellent discussion of this most challenging facial injury.

The first 7 chapters regarding concepts of rigid fixation present an excellent overview of surgical metals and specific rigid fixation applications.

This text does not fill a large void for a typical otolaryngologist. However, it should be useful to those practicing rhinology who may encounter a severe nasoethmoid-orbital fracture that does not respond well with closed reduction and external splint fixation. For the otolaryngologist who does not specialize in craniomaxillofacial surgery, this compilation of detailed information makes a useful source of reference.

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Development of Auditory and Vestibular Systems 2

R. Romand, editor. Second edition. Hard cover, illustrated, indexed, 557 pages, 1992. Elsevier Science Publishers, Amsterdam, the Netherlands, $192.50.

In considering the oncogenesis of the auditory and vestibular systems, George L. Streeter, Theodore H. Bast, and Barry J. Anson come to mind as 20th-century pioneers. These 2 systems are now among the most rapidly unfolding branches of neuroscience. This text is intended to augment the first edition (1983) by presenting data gleaned from the most recent studies and research into the morphological changes relevant to the onset of a specific function during oncogenesis; human application of morphological and functional changes seen in animals; and the type and rate of growth factors in acoustic receptors and identification of their connecting neurons. Attempts to make a tonotopic map of all levels of the auditory system are also presented to display the impact of environmental stimulation on the development of sound recognition.

The auditory system consumes 80% of this book's 17 chapters, and the vestibular system the remaining 20%. The 30 articles are essentially equally divided between North American and European contributors. The combination of their research experiences and their vast review of the literature allow for a complete panorama of the 2 systems covered.

A better understanding of the embryology of the components of the auditory and vestibular systems has been made possible by advances in such fields as molecular biology and immunohistochemistry, and the development of new tools and technology. Proteins can now be marked to serve as developmental indicators in these systems. Prior to this century it was discovered that electricity provoked the activity of the nerves and brain. Now, electrophysiologic techniques, combined with dye markings, show a response on the remodeling of neuronal processes connected with nerve structures on higher auditory centers.

Those involved in research of the auditory or vestibular system will find this text invaluable. For the clinical physician, there are timetables of events in the development of the cochlea that can lead to specific auditory defects. Also, there are reminders of environmental influences, such as ototoxic drugs and noise trauma, to which the extremely immature auditory system of the human fetus is particularly vulnerable during the second and early third trimesters of gestation. Genetic abnormalities are known to cause a variety of auditory defects. It has been observed that a lack of strial melanocytes is associated with stria vascularis dysfunction, resulting in reduced endocochlear potential. The ensuing deafness in known to occur in a number of adult white cats and dalmatian dogs, and some humans who present a delayed, progressive, sensorineural hearing loss.

Other pertinent general features of development, function, genetics, and plasticity are covered in this text. No explanation was offered for the continued progression of a congenital sensorineural hearing loss that occurs years after its initial onset.

This book makes it obvious that rapid progress in understanding the auditory and vestibular systems has been made during recent years, and that continued research and experimentation are needed at all levels of analysis. It is an expedient publication, coming at a time when knowledge of the auditory and vestibular systems has reached a point at which a review of recent and current research will be of great service to those interested in them. The text is well written in correct English, and printed on acid-free paper. It is recommended to researchers and clinicians alike.

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Diagnostic Bronchoscopy — A Teaching Manual

Peter Stradling. Sixth edition. Hard cover, illustrated, indexed, 185 pages, 1991. Churchill Livingstone, New York, NY, $125.

In conveying simply and clearly what this book is about, Dr

Stradling's excellent introduction to it could not be exceeded. He begins with an assessment of his subject matter: "While nothing can replace personal clinical practice and experience, access to a representative collection of illustrations helps to solve the problem. Normal anatomy and a variety of pathological conditions can be studied in a few hours to give preparatory knowledge, albeit superficial, which would take months to obtain in any other way. Rescrutiny of illustrations, after lesions have been seen in reality, further helps to consolidate knowledge and experience. The colour photographs reproduced in this book, forming a representative collection of normal and common abnormal findings, were all taken during routine bronchoscopy sessions and have been selected with the learner's needs in view."

Dr Stradling then stresses the aim of his book. "It is strictly limited. Technique is discussed only in outline on the assumption that a practical apprenticeship will be served. No attempt is made to produce an exhaustive atlas, to review literature, to deal with the special problems of bronchoscopy in children, to discuss the complicated subject of foreign body removal, or to advise on available apparatus . . . The book is produced to help the student of bronchoscopy appreciate the true value of the investigation and learn his way about the normal and abnormal bronchial tree; it is not an advanced textbook." Finally, the author states, "It is more important that the learner should thoroughly understand normality, and the common pathological conditions, than become confused by rarities. A few unusual conditions are included to whet the appetite."

This atlas-form book offers a fascinating panorama of bronchoscopically shown anatomy and diseases in photographs. They are skillfully presented for a learning experience. The written accompaniment and diagrams may seem overly explicit, as the photographs are so expressive.

Following the introduction, the topic is sorted by subject matter into 9 sections that include indications for bronchoscopy, fiberoptic bronchoscopy, rigid tube bronchoscopy, normality, inflammatory and associated changes, bronchial distortion and displacement, tumors, taking specimens, and miscellaneous conditions. In general, the author shows a thorough and perceptive knowledge of bronchoscopy in this small but aesthetic volume.

This book lends support to the maxim that "a picture is worth a thousand words." If only scanning the text, the novice practitioner and resident will surely learn from studying the remarkable photographs, and the seasoned bronchoscopist will have an invaluable review.

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Vestibular and Brain Stem Control of Eye, Head and Body Movements

Hiroshi Shimazu and Yoshikazu Shinoda, editors. Hard cover, illustrated, indexed, 466 pages, 1992. Japan Scientific Societies Press, Tokyo, Japan, and S Karger, Basel, Switzerland, $264.

Presentations to the Bárány Society typically represent works involving the vestibular system. This book is the result of a satellite symposium held in the late spring of 1990, at the time the Bárány Society met in Tokyo. The satellite group consisted of contemporary scientists who addressed the vestibular and brain stem control of the eye, head and body. Since it took over 2 years to publish these 37 papers, the editors obviously had to overcome some obstacles.

Symposiums that result in papers such as these provoke thought on new perplexities and provide perspectives for future research. This meeting did not solve a great number of problems, but perplexities surrounding the vestibular system were shared among kindred spirits.

Electrophysiologic tests were slowly developing by the time Rudolf Magnus and Adriaan P. H. A. de Kleijn completed their work on eye, head, and body movements in the 1920s, and electronystagmography was introduced following World War II. In the late 1970s these tests were being refined by computational science. In contrast, the basic science aspects of this field, for the most part, lagged behind. In recent years, however, vestibular research has embraced such basic aspects as developmental neurobiology, histochemistry, and membrane physiology. Today, physiologic testing and basic science studies of the vestibular system are converging.

The papers in this book are grouped into 6 sections. Section I consists of 9 papers on the functional organization of vestibulo-ocular and vestibulospinal reflexes. Discussed are the properties, patterns, and neurons involved in reflex pathways from the labyrinth to the brain stem, spinal column, and neck muscles.

Section II covers the neural mechanisms of saccade generation in association with saccadic activity, introducing such terms as pause neurons and inhibitory burst neurons. Neurons play important roles in controlling the quick phase of nystagmus.

Section III explores gaze during active head and eye saccades that orient a stationary subject to targets. The next section (IV) shows how information is carried from the vestibular structures and from the pons and medulla to specific sites in the cerebellum.

In Section V, the nervous functions executed by the basal ganglia and cerebral cortex are analyzed. Of great interest in this section is the paper that proposes that in primates there are 2 vestibulo-ocular reflexes operating independently to compensate for rotational and translateral disturbances of the head.

The last section presents a group of 4 papers dealing with functional compensation.

As seems typical of publications on this subject, pages are frequently cluttered with a myriad of term abbreviations, and many clinical entities seem to be enigmatic. However, the text is well written and the book is well organized. The research or clinical vestibuologist should find this book informative and thought-provoking.

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Surgical Anatomy of the Temporal Bone

James A. Donaldson, Larry G. Duckert, Paul M. Lambert, and Edwin W. Rubel. Fourth edition. Hard cover, illustrated, indexed, 546 pages, 1992. Raven Press, New York, NY, $130.

The first edition of this well-known monograph was introduced in 1967. The second edition, an expanded version of the first, appeared in 1973. This fourth edition follows the third edition by 11 years. It, too, is an expanded edition of its immediate predecessor, with the addition of the anatomy related to neurotology and base of skull surgery. Also included are new collaborators.

Each of the 4 sections (The Temporal Bone; The Ear: Developmental Anatomy; The Ear: Adult Anatomy; and Dissection Guide) remains under the jurisdiction of the same author-editor as in the previous editions. It is a tribute to James A. Donaldson that along with Barry J. Anson, he not only introduced this full spectrum of otologic anatomy while at the University of Iowa, but that he also continued such a tradition of excellence.

Approximately 70% of the text is made up of the section The Ear: Adult Anatomy. Some illustrations are color highlighted, breaking the monotony of serial black-and-white illustrations. There is exhaustive coverage of the squamous, mastoid, petrous, and tympanic parts of the temporal bone, which is enhanced by illustrations. Emphasis on structures and terminology, such as Hyrtl's fissure, Trautmann's triangle, and Körner's septum, would have appealed to me.

The authoritative style of the artist, George Buckley, emphasizes the surgical importance of each anatomic detail. His 2-dimensional masterpieces of the bare temporal bone give a 3-dimensional picture. Mr Buckley developed this technique with the first edition in 1968, but did not live to finish the fourth edition. The work of the new medical artist blends in very well with that of Mr Buckley, however, and it is these illustrations that make this book particularly useful for the student and clinician.

The Dissection Guide, which serves as a basic introduction to surgical dissection of temporal bones, did not appear in previous editions of this text. To perceive the dissections, the reader must refer back and forth from the guide to the book; this does not foster continuity of thoughts.

Several anatomic points became quite clear to me after seeing them depicted in this volume. For the clinician, this book would be an excellent clinical and research reference. For the student, it would be an invaluable aid to the learning of the complex anatomy of the temporal bone.

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Surgery of the Ear and Temporal Bone

Joseph B. Nadol, Jr, and Harold F. Schuknecht, editors. Hard cover, illustrated, indexed, 480 pages, 1993. Raven Press, New York, NY, $165.

It has been historically demonstrated that the greatest achievements in medicine, and particularly surgery, have been the results of diligent and thorough basic science investigations, particularly in anatomy, pathology, and physiology. The editors of this beautifully illustrated book obviously subscribe to that philosophy. They admit to having limited the contributors (26) to the text to those who received training at the Massachusetts Eye and Ear Infirmary to ensure philosophical consistency. That fact alone also ensures the readers of benefits provided by studies from one of the world's largest collections of temporal bones.

The approach to each topic is appealingly logical and simple: Here is the problem. What are the possible solutions? What are the pros and cons of each solution? In this manner, otologic problems are dealt with in 11 sections with a total of 39 chapters. Beginning with the initial ear examination, the reader is taken through surgical management of soft tissue procedures, osseous approaches to the temporal bone, and surgery for disorders involving the ear canal, tympanic membrane, middle ear, mastoid, and skull base. Customary coverage is given conditions that are usually or not uncommonly confronted by the otologist (otitis media [chronic and with effusion], otosclerosis, cholesteatoma, vertigo, perilymphatic fistula, temporal bone trauma and tumors, congenital auricular malformations, etc). The text is unique in presenting photography of pathologic findings and less frequently found information on conditions such as skin grafting, osteoradionecrosis of the temporal bone, dural defects, and others, and in providing good exposure to surgery of the external auditory canal.

The possibilities in diagnostic imaging are rapidly increasing, as evidenced in the relatively recent and current outpouring of new techniques. Some of the applications of these are discussed in Imaging of the Temporal Bone. The benefits of advancements in imaging may be readily absorbed by the otologist, and toward this end it would have been helpful to compare findings in differential diagnoses, such as an internal canal versus a posterior fossa lesion, or internal auditory canal lesions (meningioma, acoustic neuroma, lipoma, vascular lesions, metastatic lesions, etc). The use of a table might have served this purpose well.

Special attention must be directed to the illustrations, both in color and black-and-white, of Robert Galla. His talent was of great value in enhancing the text and projecting the goals of the authors.

The editors have garnered a text on the surgical treatment of ear diseases based on the pathology involved. The thoroughness of their efforts is reflected in their broad coverage of ear surgery, including all aspects of the evaluation and selection of surgical candidates and the inherent properties of ear diseases and specific surgical procedures. It would behoove anyone studying or treating diseases of the ear and, most certainly, anyone performing otologic surgery to have this book among his or her armaments.

Dennis G. Pappas, MD
Birmingham, Alabama

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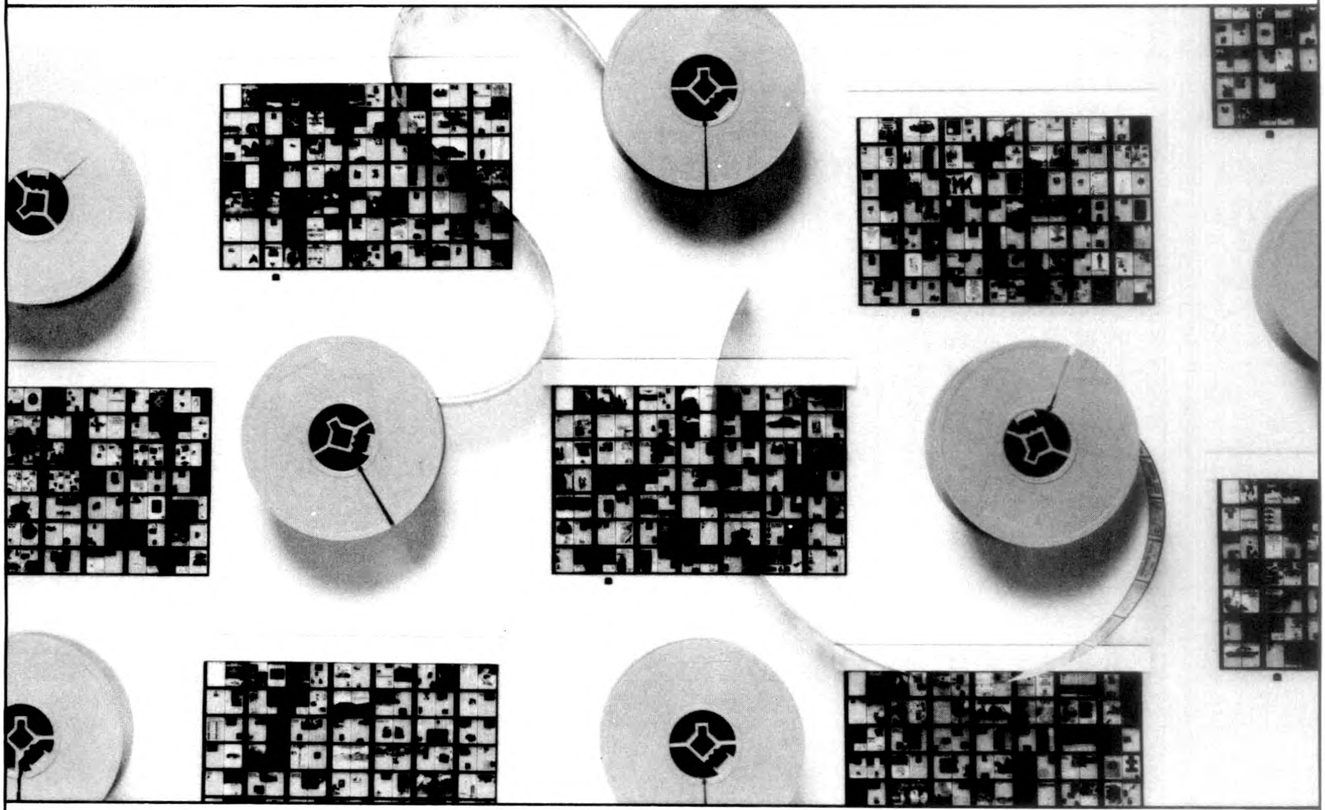

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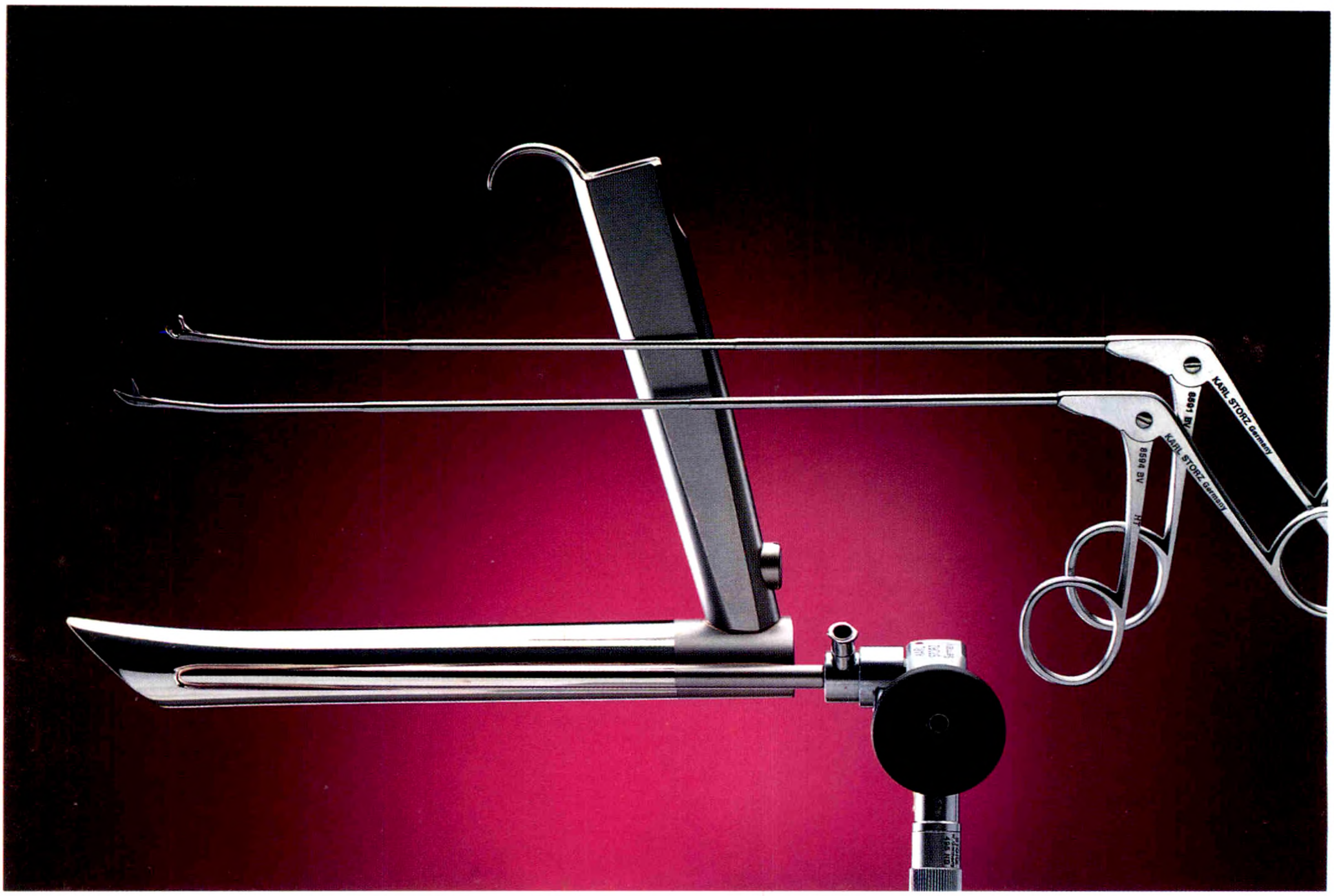

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Other drugs known to inhibit hepatic metabolism should be coadministered with caution until definitive interaction studies can be completed. The number of subjects who concomitantly received macrolide antibiotics, cimetidine, ranitidine, or theophylline along with CLARITIN Tablets in controlled clinical trials is too small to rule out possible drug-drug interactions. There does not appear to be an increase in adverse events in subjects who received oral contraceptives and CLARITIN Tablets compared to placebo.

Carcinogenesis, Mutagenesis, and Impairment of Fertility: In an 18-month oncogenicity study in mice and a 2-year study in rats, loratadine was administered in the diet at doses up to 40 mg/kg (mice) and 25 mg/kg (rats). In the carcinogenicity studies, pharmacokinetic assessments were carried out to determine animal exposure to the drug. AUC data demonstrated that the exposure of mice given 40 mg/kg of loratadine was 3.6 (loratadine) and 18 (active metabolite) times higher than a human given 10 mg/day. Exposure of rats given 25 mg/kg of loratadine was 28 (loratadine) and 67 (active metabolite) times higher than a human given 10 mg/day. Male mice given 40 mg/kg had a significantly higher incidence of hepatocellular tumors (combined adenomas and carcinomas) than concurrent controls. In rats, a significantly higher incidence of hepatocellular tumors (combined adenomas and carcinomas) was observed in males given 10 mg/kg and males and females given 25 mg/kg. The clinical significance of these findings during long-term use of CLARITIN Tablets is not known.

In mutagenicity studies, there was no evidence of mutagenic potential in reverse (AMES) or forward point mutation (CHO-HGPRT) assays, or in the assay for DNA damage (Rat Primary Hepatocyte Unscheduled DNA Assay) or in two assays for chromosomal aberrations (Human Peripheral Blood Lymphocyte Clastogenesis Assay and the Mouse Bone Marrow Erythrocyte Micronucleus Assay). In the Mouse Lymphoma Assay, a positive finding occurred in the nonactivated but not the activated phase of the study.

Loratadine administration produced hepatic microsomal enzyme induction in the mouse at 40 mg/kg and rat at 25 mg/kg, but not at lower doses.

Decreased fertility in male rats, shown by lower female conception rates, occurred at approximately 64 mg/kg and was reversible with cessation of dosing. Loratadine had no effect on male or female fertility or reproduction in the rat at doses of approximately 24 mg/kg.

Pregnancy Category B: There was no evidence of animal teratogenicity in studies performed in rats and rabbits. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, CLARITIN Tablets should be used during pregnancy only if clearly needed.

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Pediatric Use: Safety and effectiveness in children below the age of 12 years have not been established.

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In addition to those adverse events reported above, the following adverse events have been reported in 2% or fewer patients.

Autonomic Nervous System Altered salivation, increased sweating, altered lacrimation, hypoesthesia, impotence, thirst, flushing.
Body As A Whole Conjunctivitis, blurred vision, earache, eye pain, tinnitus, asthenia, weight gain, back pain, leg cramps, malaise, chest pain, rigors, fever, aggravated allergy, upper respiratory infection, angioneurotic edema.
Cardiovascular System Hypotension, hypertension, palpitations, syncope, tachycardia.
Central and Peripheral Nervous System Hyperkinesia, blepharospasm, paresthesia, dizziness, migraine, tremor, vertigo, dysphonia.
Gastrointestinal System Abdominal distress, nausea, vomiting, flatulence, gastritis, constipation, diarrhea, altered taste, increased appetite, anorexia, dyspepsia, stomatitis, toothache.
Musculoskeletal System Arthralgia, myalgia.
Psychiatric Anxiety, depression, agitation, insomnia, paroniria, amnesia, impaired concentration, confusion, decreased libido, nervousness.
Reproductive System Breast pain, menorrhagia, dysmenorrhea, vaginitis.
Respiratory System Nasal dryness, epistaxis, pharyngitis, dyspnea, nasal congestion, coughing, rhinitis, hemoptysis, sinusitis, sneezing, bronchospasm, bronchitis, laryngitis.
Skin and Appendages Dermatitis, dry hair, dry skin, urticaria, rash, pruritus, photosensitivity reaction, purpura.
Urinary System Urinary discoloration, altered micturition.

In addition, the following spontaneous adverse events have been reported rarely during the marketing of loratadine: peripheral edema; abnormal hepatic function, including jaundice, hepatitis, and hepatic necrosis; alopecia; seizures; breast enlargement; erythema multiforme; and anaphylaxis.

OVERDOSAGE

Somnolence, tachycardia, and headache have been reported with overdoses greater than 10 mg (40 to 180 mg). In the event of overdosage, general symptomatic and supportive measures should be instituted promptly and maintained for as long as necessary.

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1. Bédard P-M, Del Carpio J, Drouin MA, et al. Onset of action of loratadine and placebo and other efficacy variables in patients with seasonal allergic rhinitis. *Clin Ther*. 1992;14:268-275.

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† Relief began in 13% of treated patients vs 4% of placebo-treated patients within 30 minutes (P=.04). At 2 hours, 65% of treated patients vs 48% of patients receiving placebo experienced relief. Distribution of onset times was significantly earlier for CLARITIN Tablets vs placebo (P=.03).

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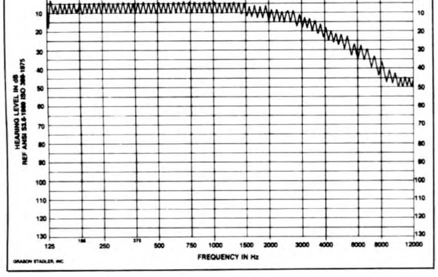

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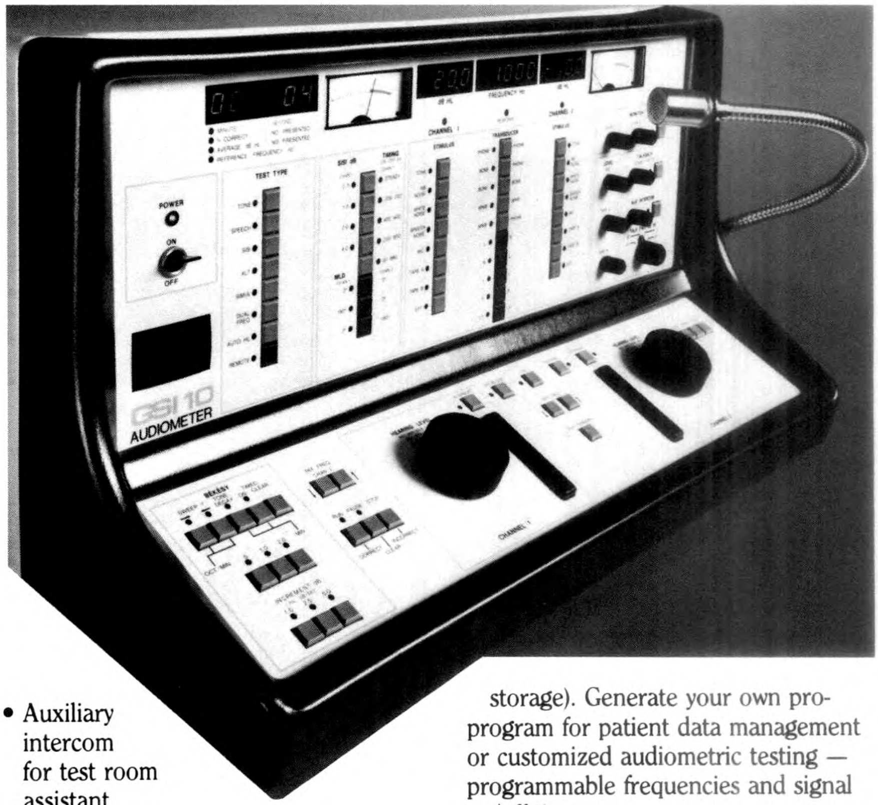

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INTERFERON ALFA-2A THERAPY FOR AIRWAY HEMANGIOMAS

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Hemangioma is a well-recognized cause of airway obstruction in the infant with stridor. Corticosteroid and laser therapy are effective in stabilizing the airway in most cases. There are, however, some extensive airway lesions that are not adequately managed by these modalities. This report describes the use of recombinant interferon alfa-2a in 15 patients with life-threatening airway hemangiomas. All patients had failed corticosteroid and/or laser therapy. Multiple upper airway sites were involved, including the base of the tongue, supraglottis, subglottis, trachea, and mediastinum. Eleven patients have completed therapy and are doing well. Four patients have resolving lesions on the drug regimen. Life-threatening airway lesions unresponsive to conventional treatment should be considered for a trial of interferon alfa-2a.

KEY WORDS — hemangioma, interferon.

INTRODUCTION

Hemangioma is the most common benign tumor of infancy, occurring in 1 of every 100 births of white children. Over half of these lesions appear in the head and neck, but the exact incidence of airway hemangiomas is not documented.[1] The airway may be involved at any level, from nose to trachea, and in some cases significant airway obstruction may ensue.

Airway lesions are most commonly found in the subglottis, and typically precipitate biphasic stridor at 6 to 8 weeks of age. About half of the infants with subglottic hemangioma also have cervicofacial cutaneous hemangiomas.[2,3] The natural history of these lesions is rapid growth for 8 to 18 months (the proliferative phase), followed by slow regression (the involutive phase) over the next 5 to 8 years.[4] Fifty percent of hemangiomas show complete regression by age 5 and 70% by 7 years, with continued slow resolution of the remaining lesions by 10 to 12 years.[5,6] It is extremely unusual for a hemangioma to spontaneously involute during the first year of life.

Many treatment modalities have been advocated for airway hemangiomas.[7] The goal of therapy is stabilization of the airway and prevention of long-term morbidity. Embolization, radiotherapy, surgical resection, and chemotherapy have all demonstrated limited success.[8-12] Corticosteroid and laser therapy have proven to be quite effective for most airway hemangiomas.[13,14] There are, however, extensive tumors that are not adequately managed by either of these modalities.

An alternative pharmacologic treatment, interferon alfa-2a, has been used recently for infants with endangering and life-threatening hemangiomas.[6] This is a report of our experience using interferon alfa-2a for obstructing airway hemangiomas unresponsive to laser excision and/or corticosteroid therapy.

METHODS

Infants with life-threatening hemangiomas of the airway were considered as candidates for treatment with interferon alfa-2a. All patients had failed corticosteroid and/or laser therapy and had progressive upper airway obstruction due to extensive lesions, involving multiple airway sites ("life-threatening"). Seven patients had also undergone tracheotomy for airway control. Eight patients in this series have been described previously[6]; they are reported here in greater detail.

Prior to starting drug therapy, a complete history and physical examination were performed. Cutaneous lesions were measured and photographed. Intra-airway lesions were documented by appropriate radiographic studies (computed tomography [CT] and magnetic resonance imaging [MRI]) and by direct laryngoscopy and bronchoscopy. Laboratory studies (complete blood count, coagulation studies, and liver enzymes) were also obtained prior to treatment, and every 3 months during interferon therapy. Interval radiographic studies were obtained as treatment progressed, every 3 to 6 months, depending on the patient's response.

Interferon alfa-2a (Hoffmann-LaRoche, specific activity 3 million U/0.5 mL) was administered subcu-

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Presented at the meeting of the American Laryngological Association, Los Angeles, California, April 17-18, 1993.

REPRINTS — Laurie A. Ohlms, MD, Dept of Otolaryngology, Children's Hospital, 300 Longwood Ave, Boston, MA 02115.

taneously at a daily dose of 3 million U/m^2 of body surface area. Each child received the initial interferon injections as an inpatient to allow close monitoring for the first 48 to 72 hours.

Evaluation of treatment response differed according to the site of lesion. Accelerated regression was defined as a 50% or greater reduction in tumor volume and was assessed by clinical status of the airway, MRI or CT, and/or endoscopy. All patients presented with stridor; clinical improvement was correlated with decreased airway symptoms. Radiographic documentation (CT or MRI) allowed pretreatment tumor volume to be measured and compared to posttreatment scans. Initial direct laryngoscopy and bronchoscopy delineated the degree of airway obstruction; on subsequent endoscopy, treatment response was recorded as percent regression, as compared to the pretreatment findings. In patients with Kasabach-Merritt syndrome (thrombocytopenic coagulopathy), stabilization of platelet count and coagulation parameters were also measured. Interferon treatment ended when the patient had achieved a 50% or greater regression in tumor size and had a stable airway.

RESULTS

Fifteen patients (13 girls and 2 boys) with life-threatening airway hemangiomas unresponsive to corticosteroid or laser therapy received interferon alfa-2a between April 1990 and March 1993. Multiple upper airway sites were involved, including the base of the tongue, supraglottis, subglottis, trachea, and mediastinum (see Table).

All patients initially presented with marked stridor due to upper airway obstruction. Seven patients had a previous tracheotomy for airway control. Fourteen (93%) had cutaneous hemangiomas as well as intra-airway lesions. A frequent pattern of hemangioma distribution was involvement of both preauricular regions, the oral cavity, and the neck.

Response to treatment was assessed by physical examination, endoscopy, and radiographic studies (see Table). Follow-up studies were chosen on the basis of each patient's status.

Eleven patients (10 girls and 1 boy) have completed interferon therapy. They ranged in age at the start of treatment from 2 to 24 months (mean 12.9 months). Duration of treatment ranged from 2 to 31 months (mean 11.3 months). Six of the 11 patients had a tracheotomy placed before interferon therapy; all 6 have been decannulated, at ages ranging from 12 to 39 months (mean 25.5 months). The remaining 5 patients in this group demonstrated accelerated regression of their lesions on clinical examination and MRI or CT scan.

Four patients (3 girls and 1 boy) continue to receive interferon alfa-2a; the duration of therapy has been 6 to 14 months (mean 10.5 months). Their ages ranged from 5 to 20 months (mean 11 months) at the start of treatment. Three patients have demonstrated gradually improving airways, and tracheotomy has been avoided. Patient 15 has a tracheotomy for continued airway control. In 2 patients (Nos. 11 and 14), large cervicofacial lesions would have made tracheotomy difficult, both technically and in terms of postoperative wound healing (Fig 1).

CASE REPORTS

Patient 1 is a 31-week premature girl who presented at age 6 weeks with biphasic stridor and failure to thrive. Physical examination revealed cutaneous hemangiomas of the scalp, abdomen, and leg. Endoscopy demonstrated hemangiomas of the subglottis (80% obstructing) and midtrachea, as well as an aberrant subclavian artery (Fig 2A). Carbon dioxide (CO_2) laser excision of the subglottic lesion was performed, and the patient was started on systemic corticosteroids (3 mg/kg per day).

Postoperatively, an MRI scan revealed that the hemangioma also involved the mediastinum and cervical spine. The patient's obstructive symptoms persisted, and 8 weeks after diagnosis she underwent tracheotomy and division of the anomalous vascular ring. Repeat bronchoscopy showed persistent airway lesions. Because of the extent of the airway hemangioma and failure to respond to corticosteroids, she began interferon therapy. Follow-up endoscopy showed 90% regression of the lesions over the next 6 months (Fig 2B). After 9 months of interferon therapy, she was decannulated, at age 12 months. At last visit (11 months after decannulation) she was asymptomatic and doing well.

Patient 8 underwent tracheotomy at age 8 weeks, at another hospital, after endoscopy revealed an extensive hemangioma involving the hypopharynx, supraglottis, and subglottis. She was transferred to our institution, where physical examination revealed additional lesions of the right parotid, the right external ear canal, and the posterior part of the neck. Over the next 15 months, both the cutaneous and airway lesions increased in size; endoscopy documented complete obstruction of the subglottis by hemangioma (Fig 3A). She began interferon therapy at age 22 months. Four months later, the airway lesions had regressed by 80% (Fig 3B). She was decannulated at age 28 months, and continues to do well.

Patient 11 presented at age 5 months with an

INTERFERON THERAPY IN FIFTEEN PATIENTS WITH AIRWAY HEMANGIOMAS

				Interferon Alfa-2a Therapy			*% Regression*	
Pt No.	*Sex*	*Site of Hemangioma*	*Previous Therapy*	*Age at Start (mo)*	*Duration (mo)*	*Clinical Outcome*	*CT/MRI*	*DL & B*
1	F	Subglottis, trachea, mediastinum, cervical spine, neck, leg	Tracheotomy, laser, steroids	3	9	Decannulated, no stridor	60%	100%
2	F	Supraglottis, base of tongue	Steroids	22	6	No stridor	No change	70%
3	F	Hypopharynx, chest, neck	Steroids	8	10	No stridor	75%	NP
4	F	Cervicofacial, mediastinum, small bowel (heart failure)	Steroids	2	31	No stridor, congestive heart failure resolved	60%	NP
5	F	Cervicofacial, subglottis (heart failure)	Tracheotomy, steroids, embolization	10	18	Decannulated, no stridor	NP	100%
6	F	Subglottis, face	Tracheotomy, steroids, laser	16	2	Decannulated, no stridor	NP	100%
7	F	Cervicofacial	Tracheotomy, steroids	24	15	Decannulated, no stridor	NP	90%
8	F	Hypopharynx, ear, subglottis, parotid	Tracheotomy	22	6	Decannulated, no stridor	NP	80%
9	F	Subglottis, vulva, buttock	Laser, steroids	7	8	No stridor	NP	20%
10	F	Subglottis, face, lip	Tracheotomy, steroids	21	9	Decannulated (28 mo), minimal stridor	NP	50%
11	M	Cervicofacial, subglottis, trachea, mediastinum	Laser, steroids	7	10	Minimal stridor	90%	100%
12*†	F	Subglottis, trachea	Steroids, cyclophosphamide	5	14	Reduced stridor	NP	NP
13*†	M	Hypopharynx, cervicofacial	Steroids	20	9	No stridor	NP	NP
14*	F	Cervicofacial, oral cavity	Steroids, laser (cutaneous)	5	6	Reduced stridor	No change	NP
15*	F	Neck, oral cavity, hypopharynx, supraglottis (Kasabach-Merritt)	Tracheotomy, steroids	14	13	Stable platelet count (Kasabach-Merritt)	No change	No change

CT/MRI — computed tomography or magnetic resonance imaging, DL & B — direct laryngoscopy and bronchoscopy, NP — not performed.

*Presently on interferon.

†Relocated; follow-up by telephone.

extensive cervicofacial hemangioma unresponsive to systemic corticosteroids (Fig 4A). He also had a cleft palate, retrognathia, and glossoptosis, consistent with Pierre Robin sequence. Direct laryngoscopy and bronchoscopy revealed bilateral subglottic hemangiomas and a lesion on the anterior tracheal wall. Carbon dioxide laser excision of the right subglottic lesion proved unsuccessful in relieving his airway obstruction. An MRI study demonstrated that the airway hemangioma also involved the mediastinum (Fig 4B). He began interferon therapy at age 6.5 months, and the corticosteroids were tapered. Follow-up endoscopy 2 months later showed slight improvement, and therapy was continued. Eight months after starting interferon (patient age 15 months), endoscopy revealed complete regression of all the airway lesions. An MRI scan and clinical examination also documented dramatic involution (Fig 5). Interferon was discontinued and the patient underwent successful repair of his cleft palate.

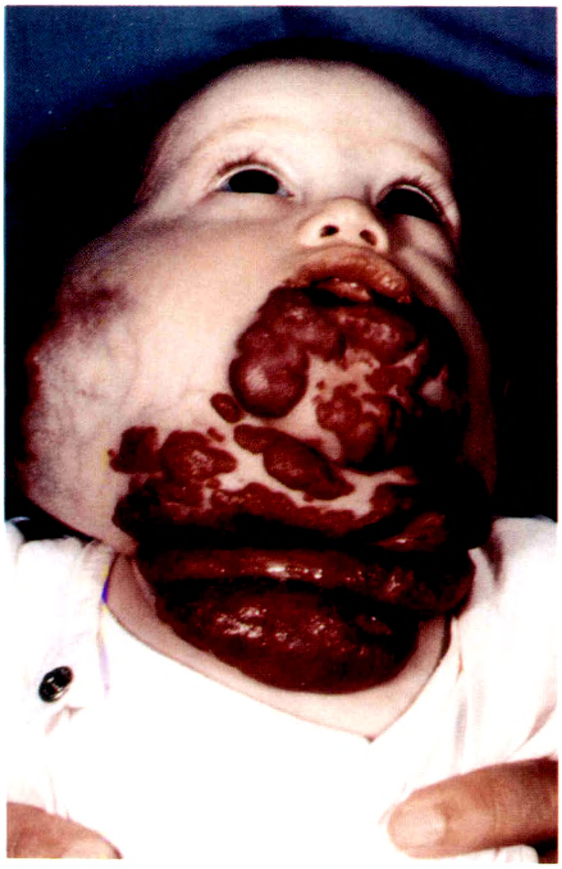

Fig 1. (Patient 14) Large cervicofacial hemangioma.

Patient 14 initially developed facial hemangioma at the age of 6 weeks; by 5 months of age, she presented with a large cervicofacial hemangioma and obstructive sleep symptoms (Fig 1). Previous treatment at other institutions included steroids and pulse-dye laser applications to the right parotid area. Direct laryngoscopy and bronchoscopy demonstrated external compression of the upper airway, with staining of the buccal mucosa; no intraluminal hemangiomas were noted. Interferon therapy was started and the steroids were tapered. Over the next 6 months, her respiratory status markedly improved and she was able to sleep supine without obstruction. A follow-up MRI scan at 6 months demonstrated a stable lesion; she continues to receive interferon. She has no stridor and is feeding well and gaining weight.

Toxicity. One patient (No. 4) demonstrated a vigorous initial response to therapy, with superficial sloughing of cheek skin. She also had a low titer (1:100) of anti–interferon alfa-2a antibodies. Interferon was discontinued, and the skin subsequently healed. Over the next 2 months, rebound growth of the hemangioma occurred and therapy was reinstituted. No other cases of rebound growth after stopping interferon were noted in this series.

All patients experienced transient low-grade fever at the start of interferon therapy. Minor elevations in liver enzymes were seen in 2 patients. No other side effects were noted. All patients are being followed up closely in a prospective fashion to identify any long-term toxicity. Thus far, weight loss has not been seen in the children receiving this therapy.

DISCUSSION

Most subglottic hemangiomas are adequately managed by either laser excision or corticosteroids. Hemangiomas that involve multiple airway sites or that cause external compression due to bulky cervicofacial disease are often resistant to conventional treatment methods. The results reported here demonstrate that interferon alfa-2a may play an important role in the treatment of these more extensive lesions.

It is important to note that the natural history of hemangioma is usually complete regression by the age of 5 to 8 years. For the majority of cutaneous hemangiomas, observation alone is preferred, while

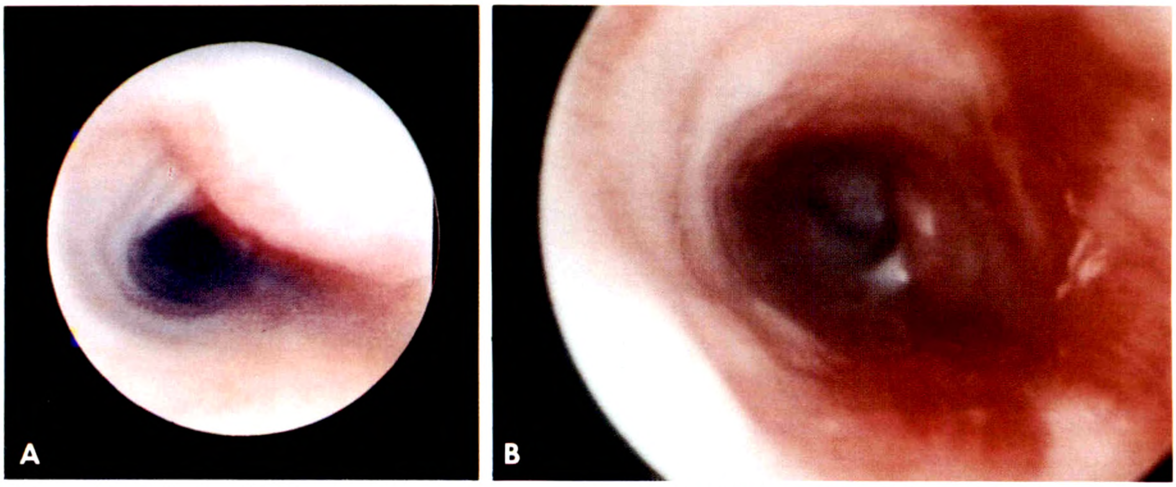

Fig 2. (Patient 1) A) Tracheal hemangioma, pretreatment. B) Residual tracheal staining, posttreatment.

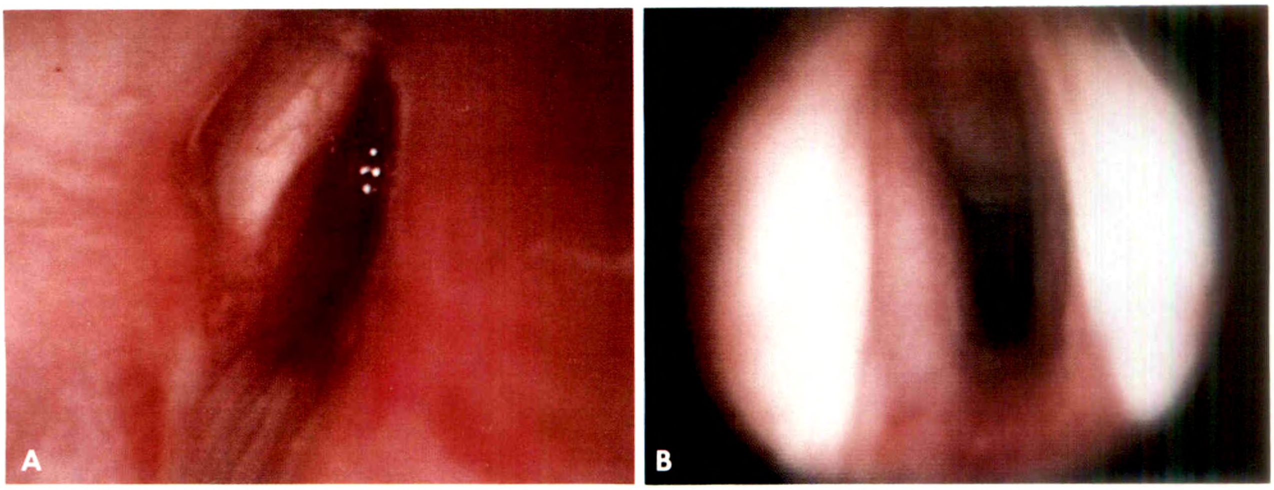

Fig 3. (Patient 8) A) Obstructing subglottic hemangioma, pretreatment. B) Subglottis, posttreatment.

awaiting spontaneous resolution.[4] Subglottic hemangiomas, however, have a mortality rate of 40% to 70% with expectant observation.[15,16] Therefore, when an airway lesion causes obstruction, intervention becomes necessary. Any treatment regimen should aim to stabilize the airway, while minimizing long-term sequelae.

Tracheotomy can secure the airway until the hemangioma regresses. The average age at decannulation for patients with isolated subglottic hemangiomas is 11 to 25 months.[2,3,8,16] In this series, patients were decannulated at an average age of 25.5 months. Our patients, however, had large airway lesions that extended well beyond the subglottis; this most likely accounts for the slightly longer cannulation time. In the past, tracheotomy alone has been associated with a mortality rate of up to 60%, with death due to obstruction by a mucus plug or accidental decannulation.[15] Therefore, other therapies have been suggested.

Sclerotherapy and cryotherapy have been used with limited success.[15] Hemangiomas are radiosensitive, but low-dose irradiation in a child carries a high risk of secondary malignancy.[8,12] Open surgical excision of isolated subglottic hemangioma has been described,[9,10] but may be associated with postoperative scarring and subglottic stenosis.

The conventional treatment for airway hemangioma is either corticosteroids and/or excision with the CO_2 laser. Many airway hemangiomas respond rapidly to systemic corticosteroids.[13,17] In a study of complicated, multisystem, life-threatening hemangiomas, Enjolras et al[18] found that 30% of patients responded

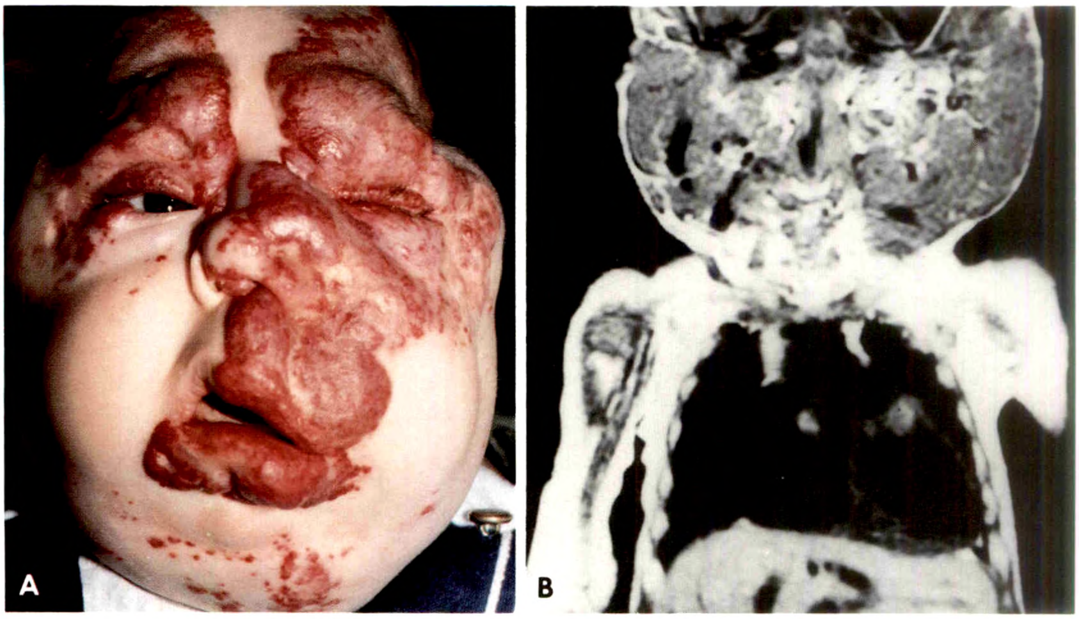

Fig 4. (Patient 11) A) Large cervicofacial hemangioma, pretreatment. B) Pretreatment magnetic resonance image.

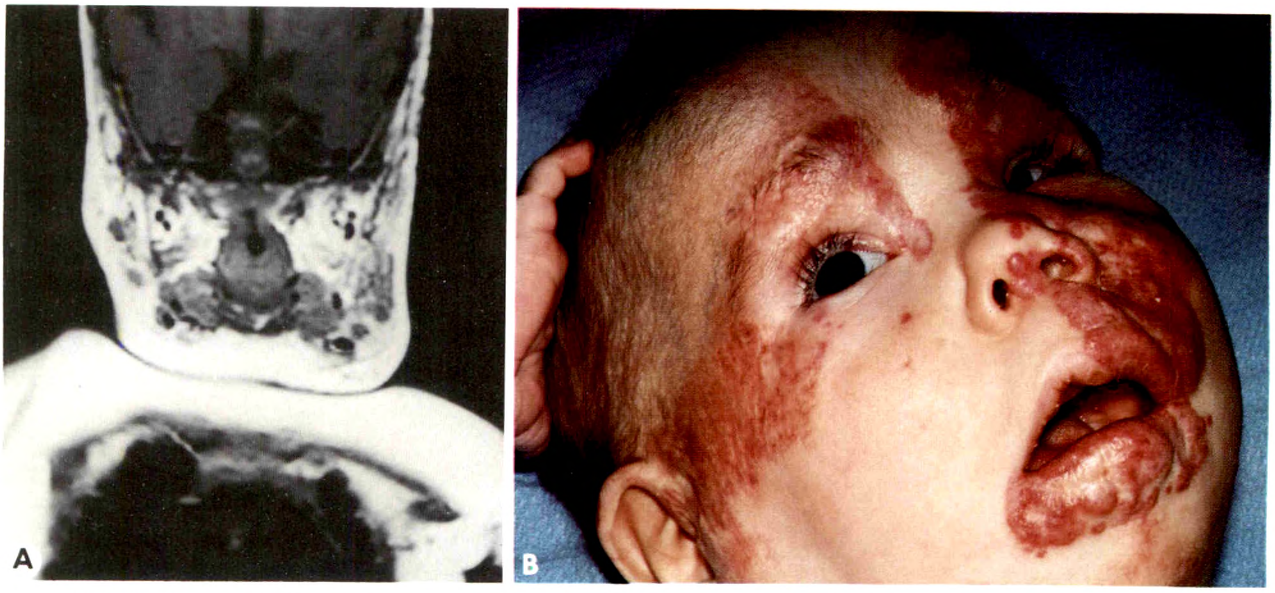

Fig 5. (Patient 11) A) Posttreatment magnetic resonance image. B) Residual cervicofacial hemangioma, posttreatment.

to prednisone. In 40%, steroids were of questionable value, and 30% of the patients showed no response at all. If a lesion is sensitive to prednisone, the effect is usually fairly rapid, within 1 to 2 weeks. Intralesional corticosteroids have also been tried in a few infants with subglottic hemangioma.[19] Long-term corticosteroid use may be associated with significant growth delay.[7]

Healy et al[20] first described use of the CO2 laser for subglottic hemangioma in 1980. Since then, numerous authors have confirmed their treatment success for unilateral, isolated subglottic lesions.[14,21] There is, however, a risk of subglottic stenosis after laser therapy, especially with excision of circumferential lesions and when multiple applications are required.[2,22] Furthermore, the CO2 laser is not effective in those patients with obstructing airway hemangiomas that extend beyond the larynx and trachea.[14] When the hemangioma involves the mediastinum, laser treatment of the intra-airway component reaches only the “tip of the iceberg.”

Extensive airway lesions that do not respond to corticosteroids or that are beyond the bounds of laser excision require other methods of management. Embolization has been used successfully in a few infants.[11] Superselective embolization, however, is difficult in the small infant and requires a skilled interventional radiologist. Risks of embolization include injury to normal tissue, sepsis, hemorrhage, and stroke.

Cyclophosphamide (along with irradiation, steroids, and embolization) has been suggested for life-threatening vascular tumors.[12,23] In treatment of a benign disease, the usefulness of this agent is outweighed by its significant morbidity, including bone marrow suppression and hemorrhagic cystitis.

Despite these various therapies, extensive life-threatening hemangiomas have a mortality rate of 20%, with death due to hemorrhage, infection, congestive heart failure, or airway obstruction.[24] A more effective therapy, with minimal morbidity, has been sought; a systemic agent that inhibits cellular proliferation of the hemangioma would be the ideal treatment.

After laboratory investigation of angiogenesis, interferon alfa-2a was chosen as a possible pharmacologic alternative for patients with life-threatening airway lesions.[6,25-27] Interferon alfa-2a was initially developed as an antiviral agent. During clinical trials in patients with acquired immunodeficiency syndrome, it was found that interferon improved Kaposi’s sarcoma, the vascular tumor associated with the human immunodeficiency virus. Early clinical reports of treatment success with interferon are detailed by Ezekowitz et al.[6] In 1989, White et al[25] reported the successful use of interferon in a 7-year-old boy with pulmonary “capillary hemangiomatosis.” Orchard et al[26] successfully treated 2 children with hemangiomas involving the abdomen and pterygopalatine space. White et al[27] reported 2 more infants with life-threatening hemangiomas treated with interferon. On the basis of these encouraging results, 20 patients with life-threatening hemangiomas (all sites) were evaluated in a prospective trial with interferon alfa-2a at our institution.[6] Eighteen patients demonstrated 50% or greater regression of their lesions in a mean of 7.8 months. Side effects were minimal, and no long-term sequelae were noted.

In its proliferative phase, a hemangioma is characterized by rapidly dividing endothelial cells. Interferon alfa-2a blocks the migration and proliferation of endothelial cells, as well as inhibiting other steps in angiogenesis[28]; thus, it is hypothesized that interferon acts as an antiangiogenic agent.[29] During angiogenesis, several growth factors have been shown to stimulate endothelial cell proliferation and differentiation; one of these important peptides is basic fibroblast growth factor (FGF).[30] It is postulated that interferon alfa-2a inhibits endothelial cell proliferation by blocking the angiogenic stimulus (ie, FGF).

One could argue that regression of these airway lesions is not a drug effect, but rather the natural history of the tumor. It is unusual, however, for a hemangioma to show spontaneous complete resolution prior to age 1 year. Therefore, as in patient 1, it would be unlikely that decannulation would be possible at 12 months of age without treatment. In this patient, interferon accelerated the regression of the hemangioma. In all patients in this series, the hemangioma continued to grow despite conventional therapy. Patient 8 had a persistent lesion with complete subglottic obstruction at age 20 months. After 6 months of interferon, the lesion regressed by 80% and she was successfully decannulated. In cases such as this, it is, of course, difficult to determine the exact cause of regression, but we suggest that interferon played a significant role. Eleven patients (11/15 or 73%) in this series achieved a 50% or greater regression of their lesion by a mean age of 21.5 months (after an average of 11.2 months of treatment). Untreated hemangiomas do not achieve complete regression until the age of 5 to 8 years, or later. Thus, we conclude that interferon hastened the resolution of the airway hemangiomas and minimized obstructive complications.

Other patients in this series presented difficult challenges in airway management. Patients 11 and 14 (Figs 1 and 4A) had extensive cervicofacial hemangiomas; tracheotomy was technically possible, but postoperative wound healing would have been problematic. With interferon alfa-2a therapy, both patients were managed successfully without tracheotomy. Literature reviews note that 54% to 67% of infants with isolated subglottic hemangioma require tracheotomy, alone or in combination with other treatment modalities.[15,16] In this series, 85.7% (6/7) of the patients with a tracheotomy were decannulated by a mean of 25.5 months. Other series report an average cannulation of 11 to 25 months (in patients with isolated subglottic hemangioma).[2,3,8,16] Thus, despite a larger tumor volume, our patients who received interferon were decannulated as quickly as patients with much smaller lesions.

Proper diagnosis is essential for effective treatment. Interferon alfa-2a inhibits endothelial cell proliferation; it is not effective in the treatment of vascular malformations.[31] In the past, proper diagnosis was made difficult by the confusing terminology used to describe vascular anomalies. At our institution, lesions are categorized according to the biologic system proposed by Mulliken and Glowacki[31] in 1982. This system is based on the cellular characteristics, anatomic features, and clinical behavior of the vascular anomaly. A lesion is classified as a hemangioma (grows rapidly in infancy, undergoes slow regression, characterized by endothelial proliferation) or as a vascular malformation (present at birth, grows commensurately with the child, normal endothelial turnover). In most patients with a vascular anomaly, a careful evaluation will permit correct diagnosis and then proper selection of therapy.

The duration of interferon therapy in this series was based on each patient's response, specifically airway stability. Early withdrawal of the medication (as in patient 4) during the proliferative phase of hemangioma development may result in rapid rebound growth and subsequent airway compromise. Prolonged therapy for 9 to 14 months is indicated in most patients.[6] As more patients are treated with interferon, the appropriate duration of drug therapy and end point criteria will become more clearly established.

Thus far, none of the patients treated with interferon at our institution have experienced any long-term morbidity. However, close follow-up is necessary to rule out any significant effects on growth and development. In the future, patients with isolated subglottic hemangiomas may be candidates for interferon therapy, but until long-term effects are established, the drug should be reserved for those patients with extensive lesions that do not respond to steroids or the CO_2 laser.

CONCLUSION

Interferon alfa-2a has been extremely effective in the management of these complicated airways. Interferon is not meant to be a replacement for traditional therapeutic modalities such as tracheotomy, steroids, and the CO_2 laser. In selected patients, however, interferon alfa-2a provides a promising alternative when conventional methods fail.

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COMPARISON OF THE CHONDROGENIC POTENTIAL OF FREE AND VASCULARIZED PERICHONDRIUM IN THE AIRWAY

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Revascularized perichondrium or periosteum may be the ideal graft to repair severe laryngotracheal stenosis because of its pliability, potential for bone or cartilage formation, resistance to infection, and rapid mucosalization. To begin our evaluation of these grafts in airway reconstruction, this study was designed to quantify the chondrogenic capacity of free and vascularized perichondrium placed in the airway. In a rabbit model, free auricular perichondrium ($N = 16$) produced a mean cartilage thickness of 0.15 mm, whereas vascularized auricular perichondrium ($N = 18$) produced a mean of 0.45 mm at 8 weeks ($p < .0001$). In a third group of rabbits ($N = 4$) vascularized auricular perichondrial grafts were subjected to a 2-hour intraoperative ischemic insult in order to simulate the ischemia of revascularization. The mean cartilage thickness at 8 weeks was 0.50 mm. We conclude that in the rabbit model, vascularized perichondrium provides significantly more cartilage than free perichondrial grafts.

KEY WORDS — laryngostenosis, perichondrium, rabbit, tracheal stenosis.

INTRODUCTION

Severe laryngotracheal stenosis is an increasingly common problem as greater numbers of persons survive airway trauma. Both endoscopic and open methods of repair have been developed, with open techniques reserved for more serious stenoses. A variety of interposition grafting materials have been used for open repairs. Some examples include hyoid bone, septal cartilage, thyroid cartilage, and costal cartilage. Under most circumstances, these grafts will be adequate for repair. However, there remains a population of patients who fail multiple procedures. These patients may have long stenoses, loss of cartilaginous support, obliteration of the airway lumen, or systemic diseases such as diabetes with poor vascularity of the recipient bed. It is for these patients that further grafting options need to be developed.

The ideal grafting material would be moldable to the shape of a lumen yet still provide rigid support. It should also be rapidly mucosalized, resistant to infection and resorption, and capable of providing an airtight seal. Vascularized perichondrium and periosteum may fulfill these criteria, because they begin as pliable grafts, but because of their inherent chondrogenic and osteogenic capacity, they should form a rigid support. Also, their inherent blood supply should make them resistant to infection and resorption.

Many studies using perichondrium and periosteum in airway reconstruction have already been performed. However, no study has evaluated vascularized periosteal or perichondrial activity when it is exposed directly to the airway, or compared its activity to that of free periosteum or perichondrium in a quantitative fashion. These issues were addressed in this study, of which the primary objective was to perform a quantitative comparison of cartilage formation in free and vascularized perichondrium placed in the airway. After the first phase of this study confirmed a significant advantage to vascularized grafts, the second phase involved the evaluation of the effect of an intraoperative ischemic insult on cartilage formation in vascularized grafts. This was performed in order to simulate the situation encountered in revascularization procedures.

MATERIALS AND METHODS

Experimental Model. The rabbit model was used, as it offers an auricular perichondrial graft that can be obtained in both free and vascularized forms. New Zealand white rabbits weighing between 3.5 and 4.5 kg were used in each of the three groups described below.

Experimental Design. Group A consisted of 18 rabbits. After anesthesia with ketamine hydrochloride (50 mg/kg) plus xylazine hydrochloride (10 mg/kg) administered intramuscularly, and intramuscular

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From the Department of Otolaryngology–Head and Neck Surgery, University of Michigan, Ann Arbor, Michigan. Supported by a grant from the American Laryngological Association. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Michigan Medical School.

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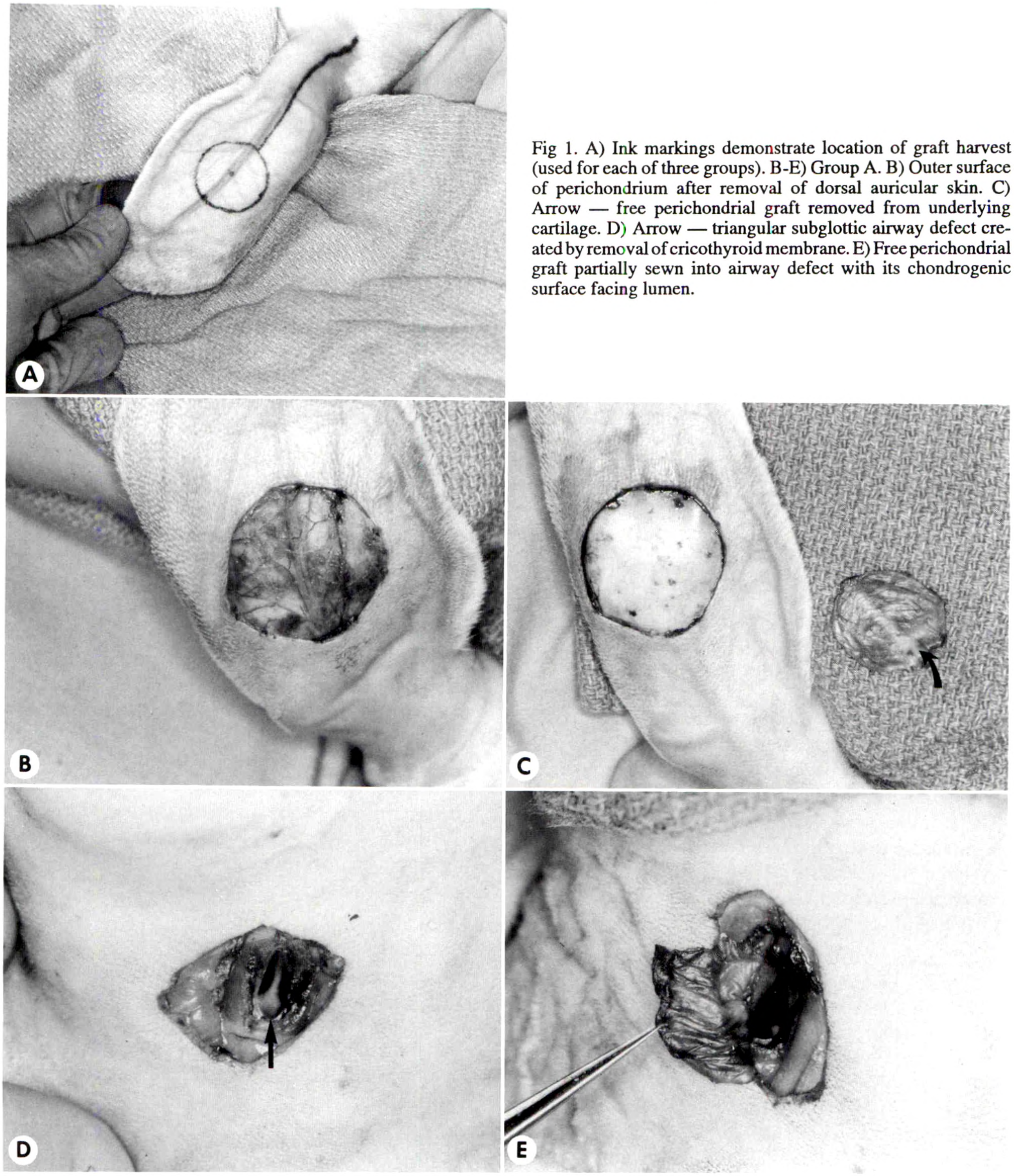

Fig 1. A) Ink markings demonstrate location of graft harvest (used for each of three groups). B-E) Group A. B) Outer surface of perichondrium after removal of dorsal auricular skin. C) Arrow — free perichondrial graft removed from underlying cartilage. D) Arrow — triangular subglottic airway defect created by removal of cricothyroid membrane. E) Free perichondrial graft partially sewn into airway defect with its chondrogenic surface facing lumen.

administration of preoperative chloramphenicol (50 mg/kg), the ears and neck of each animal were shaved, washed with Betadine solution, and draped in a sterile fashion. Next, a 2.5-cm disk of free auricular perichondrium was harvested from the dorsal surface of the right auricle with its central point located distally at two thirds the auricle's length when measured from the root of the auricle (Fig 1A-C). A midline neck incision was used to expose the airway. The cricothyroid membrane was then removed to create a triangular airway defect in the anterior subglottis (Fig 1D). The free perichondrial graft was then placed across the defect with the chondrogenic surface facing the airway. The graft was secured around the perimeter of the defect with multiple interrupted 4-0 Vicryl sutures to obtain an airtight seal (Fig 1E). The neck

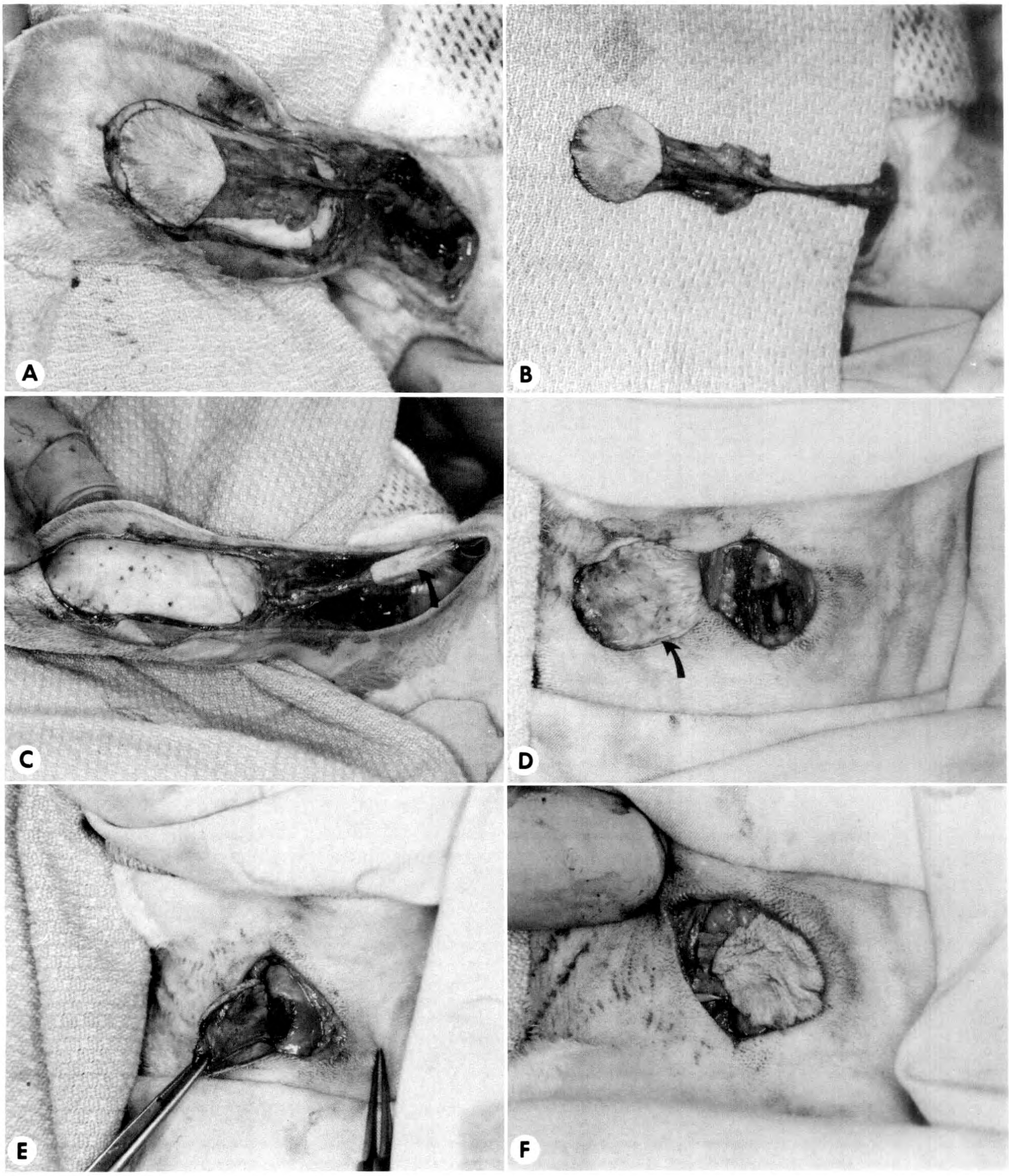

Fig 2. Group B. A,B) Pedicled auricular perichondrocutaneous graft harvest. C) Arrow — pedicled graft being pulled through subcutaneous tunnel connecting ear and airway. D) Arrow — perichondrium of pedicled graft ready for placement in airway. E) Pedicled graft partially sewn into airway defect. F) Pedicled graft completely sewn into airway defect with its overlying skin now visible for postoperative viability monitoring.

wound was then closed primarily. The cartilage and attached ventral skin of the auricular donor site were removed to create a circular auricular defect with no exposed auricular cartilage. Each animal was then recovered and maintained for 8 weeks prior to painless sacrifice.

Group B consisted of 24 rabbits. After anesthesia, shaving, Betadine wash, and sterile draping as in group A, each animal had an auricular perichondro-

TABLE 1. AVERAGE CARTILAGE THICKNESS IN MILLIMETERS THROUGH 20 SECTIONS AT 8 WEEKS FOR ALL GROUPS

Animal No.	*Group A (N = 16)*	*Group B (N = 18)*	*Group C (N = 4)*
1	0.14	0.53	0.46
2	0.09	0.50	0.59
3	0.00	0.21	0.34
4	0.05	0.62	0.61
5	0.39	0.08	
6	0.08	0.43	
7	0.00	0.42	
8	0.31	0.66	
9	0.46	0.65	
10	0.12	0.09	
11	0.00	0.49	
12	0.05	0.52	
13	0.15	0.19	
14	0.02	0.49	
15	0.39	0.40	
16	0.13	0.67	
17		0.61	
18		0.54	
Mean	0.15	0.45	0.50

cutaneous graft measuring 2.5 cm in diameter harvested from the right auricle. The location of the graft harvest was the same as for group A; however, these grafts also included the overlying dorsal auricular skin and were attached to a vascular pedicle consisting of the central auricular artery and vein. This pedicle was dissected free to the root of the auricle to allow adequate length for rotation into an airway defect (Fig 2A,B). Next, a midline neck incision was used to expose the airway and a defect was again created by removal of the cricothyroid membrane. The graft and its pedicle were then passed through a subcutaneous tunnel connecting the root of the auricle and the airway defect site (Fig 2C,D). The graft was then placed across the defect as in group A and secured around the defect perimeter with interrupted 4-0 Vicryl sutures (Fig 2E,F). The skin edges of the graft were then approximated to the skin of the midline neck incision. In this way, the skin of the vascularized graft could be evaluated for viability postoperatively. Wound closure, recovery, and postoperative care were identical to those in group A, except that serial examination of the skin paddle was used to assess graft viability. The animals were sacrificed painlessly at 8 weeks.

The 4 rabbits in group C were treated identically to those in group B with one exception. Prior to rotation into the cricothyroid membrane defect, the central auricular artery and vein were clamped for a period of 2 hours in order to simulate the conditions of a revascularization procedure. Recovery, postoperative care, and painless sacrifice at 8 weeks were managed as in the other two groups.

Complications. Within each group, some animals developed wound infections. In group A, 5 animals developed infections, 3 at the auricular site and 2 at the neck site. In group B, 10 animals developed infections, all of which occurred at the auricular site. This large number of auricular infections in the group B animals was noted early on in this study and felt to result from a dissection practice that included denuding auricular cartilage at the base of the auricle when releasing the auricular vascular pedicle. Correcting this small point in the pedicle dissection eliminated auricular infections thereafter. In group C, 1 animal developed an infection in the left foot for unknown reasons. All infections were treated with debridement and topical silver sulfadiazine ointment applied twice daily. Wound infections were transient in all but 7 animals: 2 from group A, 4 from group B, and 1 from group C. Therefore, these 7 animals were sacrificed early and not included in further evaluation. In addition to wound infections, 1 animal from group B developed obvious venous congestion and ischemia of the skin indicator paddle of its auricular perichondrocutaneous graft on the second postoperative day. This was felt to occur secondary to thrombosis of the venous pedicle, and this animal was also sacrificed early and not included in further evaluation. Last, 1 animal from group B died spontaneously of unknown causes on postoperative day 7.

Data Analysis. After sacrifice, the airways were harvested and formalin-fixed. The subglottic airway, which included the defect and overlying graft, was then embedded in paraffin. Sections at 5-µm intervals were obtained with a microtome. Each section was then stained with hematoxylin and eosin. Next, 20 sections taken at serial intervals throughout the length of the defect and graft were used for evaluation. These 20 sections were not examined prior to selection in order to eliminate a bias in selecting optimal cuts. Each section was then evaluated with a digitometer program (Imagescan V3.0 system) that allowed measurement of the area of neocartilage across the airway defect, as well as the width of the defect. Division of the area by this width then gave the mean cartilage thickness for each section. With this method, thickness measurements were much more representative of their sections than would be a single measurement taken at some arbitrary point in each section. The 20 thickness measures for each animal were then averaged. These results are shown in Table 1.

RESULTS

A mean cartilage thickness of 0.15 mm was found

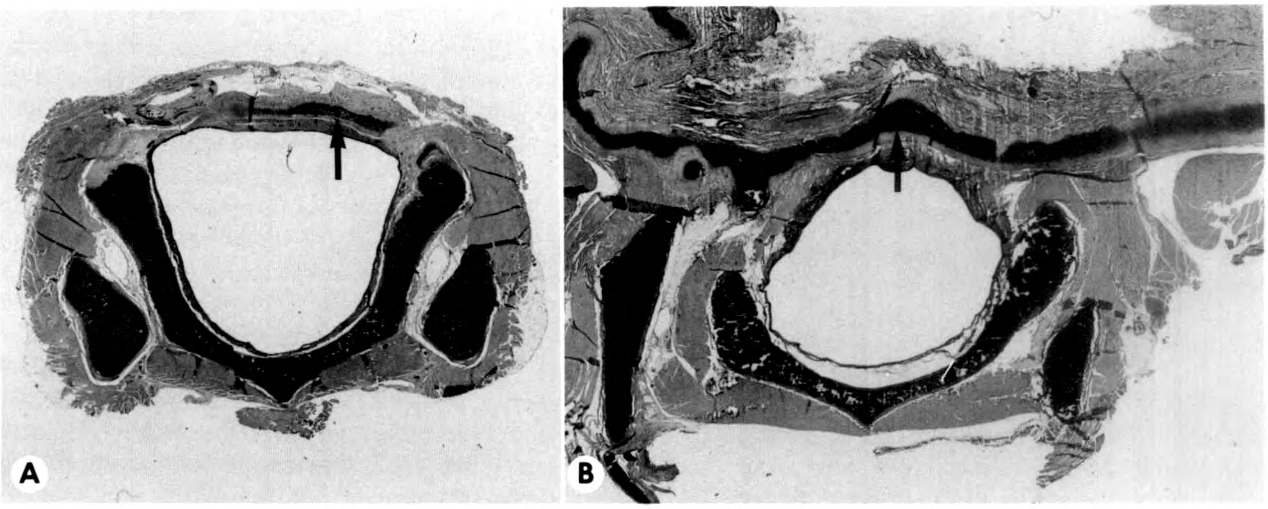

Fig 3. Cross sections of subglottic airway. A) Group A animal. Average thickness in this section was 0.12 mm. Arrow — shard of cartilage that has formed from free perichondrial graft. B) Group B animal. Average thickness in this section was 0.49 mm. Arrow — new cartilage that has formed from vascularized perichondrial graft.

in the group A airway defects covered with free auricular perichondrium, whereas the mean thickness in those defects covered with vascularized perichondrium (group B) was 0.45 mm. Figure 3 demonstrates sections from group A and B grafts illustrating this difference. Figure 4 illustrates that the cartilage thickness in both groups A and B is bimodally distributed. Only 25% of group A grafts produced an average thickness of more than 0.3 mm. In contrast, 78% of those in group B produced at least 0.3 mm of cartilage. Analysis of this difference was performed with the Wilcoxon rank sum test. This demonstrated a highly statistically significant difference between the groups ($z = 3.97$, $p < .0001$). It appears that in this model, cartilage production is either minimal or substantial. The study strongly suggests that providing a blood supply to the grafts produces a much greater likelihood that substantial cartilage formation will occur.

Within group A the 3 animals with transient wound infections had an average cartilage thickness of 0.11 mm. Within group B, the average cartilage thickness in the 6 animals with transient wound infections was still 0.49 mm. Because the differences between those infected and those without infection are small, all animals have been included in the comparison evaluations. In regard to our second objective, the grafts in group C, subjected to a 2-hour intraoperative ischemic insult, produced a mean cartilage thickness of 0.50 mm. A bimodal distribution was not noted in this small group.

DISCUSSION

Many studies have been performed using periosteal and perichondrial grafts in laryngotracheal re-

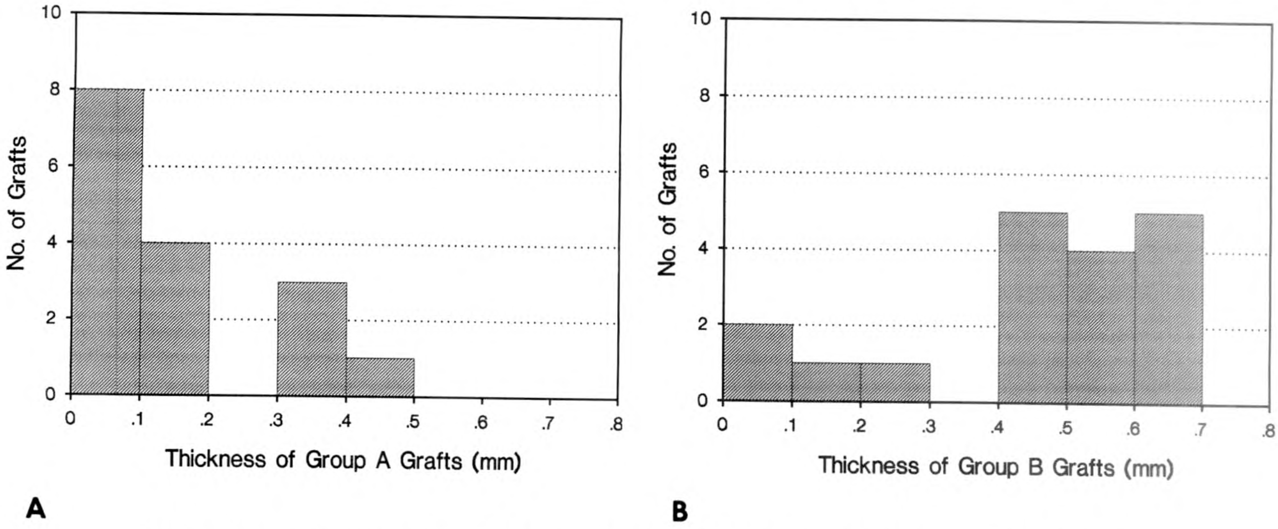

Fig 4. Histograms demonstrating distribution of cartilage thickness in grafts. A) Group A. B) Group B.

TABLE 2. LARYNGOTRACHEAL RECONSTRUCTION USING PERIOSTEAL OR PERICHONDRIAL GRAFTS

Authors	*Year*	*Model*	*Donor Site*
Ritsala and Alhopuro[1]	1973	Rabbit	Tibia
Sohn and Ohlsen[2]	1974	Rabbit	Auricle
Ohlsen and Nordin[3]	1976	Rabbit	Auricle
Rice and Coulthard[4]	1978	Dog	Thyroid cartilage
Cohen et al[5]	1985	Pig	Tibia
Fonkalsrud and Plested[6]	1966	Dog	Rib
Krespi et al[7]	1983	Dog	Rib

construction. Table 2[1-7] summarizes certain features of these studies. In animal studies employing free periosteum and perichondrium in the airway, bone and cartilage formation are reported, but quantification is lacking.[1-5] Cohen et al[5] found that free tibial periosteal grafting provided sufficient support to bridge a 30% defect of the airway circumference, but when the defect increased to 50%, the airways lacked adequate support and collapsed. In contrast, Fonkalsrud and Plested[6] and Krespi et al,[7] using pedicled and revascularized pleuroperiosteum, have demonstrated good bone formation in tracheal and subglottic reconstructions bridging complete circumferential defects. However, these studies[6,7] using vascularized pleuroperiosteum bypass the effect of contamination, as pleura serves as a barrier between the periosteum and airway secretions. They demonstrated that free periosteum and perichondrium can form bone or cartilage in a contaminated environment such as the airway, although it may be inadequate for repair of extensive defects, and that revascularized periosteum in a sterile environment can form enough bone for complete airway support.

This study has confirmed that in a rabbit model, vascularized perichondrium can form substantial amounts of cartilage when applied directly to the airway, that vascularized perichondrium forms significantly more cartilage than free perichondrium, and that vascularized perichondrium does not appear to be damaged by a 2-hour intraoperative ischemic insult.

The use of perichondrial and periosteal grafts in humans thus far has been limited. Despite the many studies involving free grafts, we have found no clinical reports of their use in humans. In 1964, Narodick et al[8] described a case involving use of a rib periosteal graft pedicled on the intercostal vessels to repair a 5 × 2-cm defect of the thoracic trachea. The graft was placed with its periosteal surface toward the tracheal lumen and its pleural surface toward the mediastinum. Narodick et al cite the graft's advantages as the ability to create an airtight seal and its rapid epithelialization. They do not comment on the osteogenic capacity of the graft, but do state that the graft was too rigid to allow biopsy at bronchoscopy performed 1 month postoperatively. The sternocleidomastoid myoperiosteal flap described by Friedman et al,[9] which should receive some blood supply from its attached muscle, has been used in both animal and human subjects. This graft is pliable, forms bone, and appears to be a suitable option for defects of the subglottic larynx. It will be limited in the repair of larger defects by the size of the sternocleidomastoid's periosteal insertion. The use of vascularized bone in the reconstruction of larger airway defects was described by Kambic et al,[10] who reported the use of revascularized epithelialized iliac crest for combined subglottic and upper tracheal reconstruction. This flap provides vascularity, firm support, and large size. However, it requires two stages to perform, the graft is bulky, and it cannot be molded to the shape of the lumen. In contrast, a graft such as revascularized iliac periosteum might provide the large size of the iliac crest graft[10] along with the moldability of the sternocleidomastoid myoperiosteal graft.[9] This graft has already been used in the successful repair of an open and comminuted fracture of the lower leg in a human subject.[11]

CONCLUSIONS

This study has demonstrated that vascularized perichondrium produces significantly more cartilage than free perichondrium when placed in the airway, and does not appear to be adversely affected by a 2-hour ischemic insult. Still, many other factors may influence the chondrogenic or osteogenic capacity of these grafts in human subjects. The age of the individual and the location of the graft donor site may be important factors. Another concern will be the effect of an underlying airway stent, an area we are currently investigating. Despite the many factors involved, the results of this study justify further evaluation of these grafts for use in laryngotracheal reconstruction.

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The 1994 Midwinter Meeting of the Association for Research in Otolaryngology will be held in St Petersburg Beach, Florida, February 6-10, 1994. For information, contact Michele Johnson, Executive Secretary, 431 E Locust St, Suite 202, Des Moines, IA 50309; telephone (515) 243-1558; fax (515) 243-2049.

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FOURTH INTERNATIONAL CONFERENCE ON HEAD AND NECK CANCER

The American Society for Head and Neck Surgery and the Society of Head and Neck Surgeons will sponsor the Fourth International Conference on Head and Neck Cancer, to be held from July 28 to August 2, 1996, in Toronto, Canada. For further information, contact We Plan Meetings, Inc, Ruth C. Enquist, President, 1503 15th St NE, Rochester, MN 55906; telephone (507) 285-1523; fax (507) 281-8328.

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LIMITATIONS OF ELECTROMYOGRAPHY AND MAGNETIC STIMULATION FOR ASSESSING LARYNGEAL MUSCLE CONTROL

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The development of new phonosurgical techniques has increased the level of interest in the field of neurolaryngology. This field requires valid techniques for determining if muscle activation is normal. Laryngeal electromyography is being used more frequently to assess muscle innervation and synkinesis. Further, magnetic stimulation has been introduced as a noninvasive technique for nerve stimulation. Technical limitations that affect the clinical utility of both these techniques are reviewed: 1) difficulties obtaining selective and accurate electromyographic laryngeal muscle recordings, 2) normal variation in movement and muscle activation patterns within and between normal individuals when producing the same speech syllables, and 3) variation in laryngeal muscle response latencies between and within normal subjects during peripheral magnetic stimulation. Given the normal variation in laryngeal electromyography and magnetic stimulation response latencies, these techniques may not yet be reliable or accurate for assessing reinnervation or synkinesis following recurrent laryngeal nerve injury.

KEY WORDS — electromyography, laryngeal muscles, magnetic stimulation.

INTRODUCTION

With the successful use of botulinum toxin in the spasmodic dysphonias, and new surgical treatments for recurrent laryngeal nerve (RLN) injury such as thyroplasty and laryngeal nerve anastomosis, interest has increased in the clinical application of laryngeal electromyography (EMG).[1-6] The development of improved treatments for patients with specific vocal fold movement abnormalities depends on improving our understanding of the actions of the laryngeal muscles for voicing control during speech. To distinguish between voiced and voiceless consonants in speech, for example, phonation must be onset and offset at rapid intervals, sometimes as short as 20 milliseconds (ms). During /b/, phonation begins during the bilabial closure, while for /p/, a 20-ms silent interval occurs before phonation begins, after release of the bilabial closure.[7] Knowledge of how the laryngeal muscles are used to achieve these rapid changes in vocal fold position for speech is still limited. Several studies addressed these issues in the 1970s by averaging across many productions of the same speech sounds in a few normal speakers to determine how laryngeal muscle activity level related to speech sound production.[8-11] Characteristic *average* patterns of laryngeal muscle activation were identified for particular voicing gestures in normal speakers. Such information might be applied to patient evaluations to determine when an abnormal or synkinetic pattern of muscle activation occurs.

The potential for clinical application of these findings merits their evaluation. An average pattern sometimes obscures the degree of variability in muscle activation for individual syllable productions, both within and among individuals. Data are needed on how consistent these patterns are between syllables within and across normal speakers before they can be used for patient evaluation.

Magnetic stimulation has recently been proposed as a noninvasive technique for stimulation of peripheral nerves, including the laryngeal nerves.[12] Because of the depth of the induced electrical field, those nerves inaccessible to surface stimulation might be studied by this technique. Percutaneous or surface electrical stimulation is not feasible as a clinical procedure in patients with suspected nerve injury, because when no response is obtained, it could be either because the nerve was not intact, or because the stimulating electrodes were not close enough to the nerve. Magnetic stimulation offered a new clinical technique for noninvasive evaluation of the RLN and superior laryngeal nerve (SLN). For such a technique to have clinical utility, however, consistent responses to magnetic stimulation must be obtained in normal subjects so that delays in nerve conduction time can be easily identified.

The purpose of this study was to provide data on normal subjects by using these 2 techniques, laryngeal EMG and magnetic stimulation, to assess their potential utility for clinical assessment of patients with vocal fold movement disorders. If these techniques are to have clinical utility, they must be reliable in normal speakers. Consistent patterns of

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laryngeal muscle patterning must occur in normal speakers to determine when patterns are abnormal for speech in patients with vocal fold movement control disorders. Second, the consistency of thyroarytenoid (TA) muscle responses during peripheral nerve magnetic stimulation will be evaluated. Here again, consistent conduction times to the TA muscle must be determined in normal adults for this technique to have utility for evaluating the integrity of the RLN in patients.

TECHNICAL LIMITATIONS IN STUDY OF LARYNGEAL MUSCLE ACTIVITY DURING SPEECH PRODUCTION

Selective Laryngeal Electromyography. To assess the activation pattern of a particular muscle during speech, selective and accurate electrode recording techniques are required. Four different electrodes are currently in use that have advantages and disadvantages for this type of study.[13] The least selective are monopolar electrodes. Because 1 of the 2 poles is a reference electrode on the neck, the recording field will pick up potentials from several muscles at a time in the larynx. These are used for injecting botulinum toxin into the laryngeal muscles and are useful for determining when an electrode is in muscle tissue, but are not useful for identifying a particular muscle. Bipolar hooked wire electrodes are most often used when lengthy recordings are needed during speech and other movements, because the hooks maintain the position within the muscle tissue during movement. They have a distinct advantage over needle electrodes in that needles will move during muscle contraction. Therefore, needles are not useful for measuring muscle activity during speech or other movements. Needle electrodes can be used for sampling a muscle, and although concentric needle electrodes have most frequently been used in clinical EMG, Dedo[14] demonstrated that these electrodes could not be used to accurately assess reinnervation. Only bipolar needle electrodes, which have both poles contained in the center core, are adequately selective to assess TA reinnervation without picking up cricothyroid (CT) muscle potentials.[14,15] Hirano et al[16] used these electrodes in a large series of patients and found them accurate for predicting reinnervation.

Accessing Individual Muscles. Bipolar hooked wires are most useful when speech or other movements are to be studied,[11] but have the disadvantage of not being highly selective. For TA recordings, needles bearing the hooked wires are inserted transcutaneously through the CT membrane, between the 2 cartilages and below the level of the target muscle. The needle is angled superiorly and laterally; this position often places the hooked wires in the posterior portion of the TA. Even with attempts to angle the electrode as superiorly and medially as possible, the point of muscle recording is often posterior, close to the vocal process of the arytenoid cartilage, where the TA and lateral cricoarytenoid (LCA) muscles insert and are close to the medial portion of the CT.[17] Coronal sections of the adult human larynx show this most clearly: for example, the coronal section at the posterior macula plava, AC1, in the histologic atlas by Hirano and Sato.[18] Therefore, TA muscle recordings using the transcutaneous approach may record motor units from all 3 muscles and may vary considerably depending on electrode placement.

In a more recent study, EMG of the TA muscle was conducted in 47 patients with vocal fold paralysis[19] following esophageal cancer surgery. They classified the recordings on whether they had 1) electrical silence or fibrillation potentials, 2) only a reduction in active motor units, or 3) a normal pattern. The percentages of patients with recovery of vocal fold movement within 6 months differed among the 3 groups; 18.7% of those with electrical silence or fibrillations recovered, while 38% of those with reduced numbers of motor units firing and 80% of those with a normal activity pattern recovered. Although these groups differed, the prediction was not always accurate, that is, of the 47 cases, errors were found when patients with electrical silence or reductions in motor units recovered, and when those with a normal pattern did not recover. The total prediction error rate was 12 of the 47 cases, or 25%.

This paper is concerned with the use of EMG for patients with vocal fold movement control disorders, including 1) patients with poor motility after reinnervation and 2) patients who have laryngeal control abnormalities as in spasmodic dysphonia, paradoxical vocal fold movement, or dyspraxia. In these patients volitional movement control is affected and assessment is aimed at determining whether movement patterning is abnormal.

Physiologically Distinct Muscle Compartments. Another difficulty affecting our understanding of the laryngeal muscles is the degree to which each of the muscles is composed of physiologically distinct compartments. Anatomic examination of portions of each of the laryngeal muscles suggests that the fiber directions, origins, and insertions differ for components of each of the laryngeal muscles. For example, the glottal and supraglottal portions of the TA muscles differ in their insertion points and fiber directions. The supraglottal portion may affect the motion of the ventricular folds, while the glottal portion is more likely to affect only the vocal fold. However, we currently have no information on whether these 2 portions are *physiologically* distinct. Similarly, the rectus and oblique portions of the cricoarytenoid muscle have long been recognized anatomically. However, we do not know whether these are physiologically distinct in humans or whether they differ physiologically from that portion of the CT that inserts into the medial surface of the thyroid cartilage.

Intramuscle Homogeneity in Motor Unit Firing Patterns. Recent studies have demonstrated that the

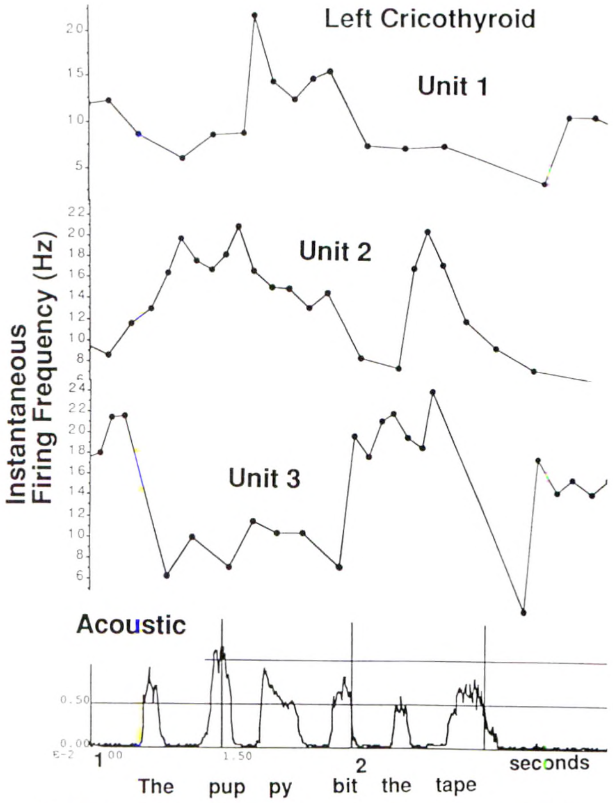

Fig 1. Simultaneous instantaneous firing frequency of 3 motor units detected in cricothyroid muscle recording in normal speaker by means of bipolar hooked wires. Digital approach to window discrimination was employed for unit detection with visual verification.[20] Instantaneous firing frequency was derived from inverse of intervals between successive unit firings. Bottom tracing is rectified presentation of simultaneous speech recording of sentence "The puppy bit the tape."

laryngeal muscles are not homogeneous in their motor unit firing patterns. In humans, using concentric needles or bipolar hooked wires to record from the TA and CT muscles during respiration, Chanaud and Ludlow[20] demonstrated that motor units within a muscle fired independently. Simultaneously within the same muscle, motor unit firings with different firing patterns were recorded from the same electrode. Some were phasically active only during inhalation, and others only during exhalation; still others were tonically active throughout respiration. Larson et al[21] obtained similar findings when recording from motor neurons in the nucleus ambiguus in the awake monkey. Five classes of motoneurons for the TA muscle were recorded from the nucleus ambiguus. The firing of 1 class was related to respiration, while a second had a complex discharge pattern that increased during vocalization and before and after vocalization. A third only fired before and during vocalization, and a fourth fired following vocalization. A fifth class reduced their discharge rate during vocalization.

In normal humans, variations in motor unit firing patterns can also be seen in a muscle during speech. In Fig 1,[20] the firing frequencies of 3 motor units recorded from 1 set of bipolar hooked wires in a normal speaker's CT muscle are plotted for the sentence "The puppy bit the tape." These units were detected by the same technique as employed by Chanaud and Ludlow.[20] The points in time at which each of the units was firing most rapidly differed. Thus, the heterogeneity of firing patterns of individual motor units within the same muscle suggests an inherent complexity in muscle activation patterns. The muscle activation pattern found during EMG may vary depending on which motor units are recorded by an electrode placed in a muscle.

Measuring Similar Movements. Another difficulty with obtaining information on the role of the laryngeal muscles in speech production is determining whether subjects performed the same vocal fold movements while speaking. To evaluate the similarity in laryngeal movement patterns used by speakers to produce the same speech syllables, we selected at random 5 videotape recordings from those performed on normal speakers in our laboratory. During each recording, subjects repeated 2 speech gestures on a single exhalation: repetitions of a syllable containing a glottal stop (/ʔ/) and the vowel /i/, and repetitions of a syllable containing the voiceless consonant /s/ and /i/. Each subject's videotape was rated by 3 examiners on the extent of motion seen in the arytenoids, the vocal folds, and the ventricular folds, during repetition of the 2 types of speech syllables (see Table). Ratings of 0 indicated no observed structure movement, 1 indicated minimal movement, and 2 indicated clearly observable movement. The subjects varied in their extent of structure movement. Although these normal speakers were producing the same speech syllables, they moved their larynges in discernibly different ways. This between-subject variation in speech movement may interfere with determining whether a patient is moving the larynx abnormally during speech. Further, if subjects move their larynges differently to produce the same speech sounds, then it is unlikely that they will use the same muscle contraction patterns to produce these sounds.

VARIATION IN MUSCLE ACTIVITY FOR SPEECH GESTURES WITHIN AND BETWEEN NORMAL SPEAKERS

Analysis of Laryngeal Muscle Activity. During speech, 4 types of vocal fold opening and closing gestures occur: 1) adduction for phonation onset to begin a vowel, 2) adduction to interrupt phonation by a glottal stop, 3) vocal fold opening from the vibratory position for a voiceless consonant such as /s/, and 4) vocal fold abduction for phonation after a glottal stop. Laryngeal muscle activity can be measured during intervals in which either vocal fold adduction or abduction is used to produce speech sounds. By comparing mean muscle activity levels during voice onset and offset, it can be determined if a muscle is more active for vocal fold adduction or for abduction.

EXTENT OF MOVEMENT OF LARYNGEAL STRUCTURES DURING NASOLARYNGOSCOPIC VIDEO RECORDINGS OF SPEECH SYLLABLE REPETITION

			Laryngeal Structures		
Speech Syllable	*Subject*	*Sex*	*Vocal Folds*	*Arytenoids*	*Ventricular Folds*
/ʔi/	A	M	Obscured	1	2
	B	F	2	2	2
	C	F	2	1	2
	D	M	2	2	0
	E	M	Obscured	1	2
/si/	A		Obscured	2	0
	B		2	2	1
	C		2	2	0
	D		2	2	0
	E		2	2	2

0 — no observed structure movement, 1 — minimal movement, 2 — clearly observable movement.

In Fig 2,[22] EMG recordings from a normal speaker who participated in a previous study are presented to illustrate the method used to measure muscle activity during particular speech gestures. In this example, mean muscle activity was measured during a 100-ms interval, including 50 ms before phonation onset and 50 ms after phonation onset. In this instance, TA activity was greater during vocal fold adduction for phonation onset than during vocal fold abduction for phonation offset. Conversely, the posterior cricoarytenoid (PCA) and CT recordings, in this instance, were greater during abduction than during adduction.

Results. To determine the consistency of muscle activation patterns during repetitions of the same speech syllable, data from 1 normal speaker, selected at random from participants in a previous study,[22] were measured. Mean muscle activity was measured from 100-ms windows during adduction and abduction, as indicated by the speech signal (Fig 2). The mean level of muscle activity was computed for each adduction and abduction window for each muscle. The speaker was repeating the syllable /si/, 6 times on a single breath (Fig 3). Although the DC component of the photoglottographic signal demonstrated a similar change in glottal area during each repetition, the muscle activity varied across syllable productions. Further, the TA and CT muscles on the 2 sides were dissimilar. Differences in activation patterns between the right and left sides of the same muscle were previously reported in 2 additional speakers.[22] The mean values for the adduction and abduction portions of each repetition by the subject were plotted for each of the 5 muscles (Fig 4). The 10 mean values were from 2 series of syllable repetitions: 1 series is shown in Fig 3 and contained 6 repetitions, and the other contained 4 repetitions.

Mean muscle activity varied considerably during the 10 segments of adduction and abduction during repetition of the same syllable by the same speaker.

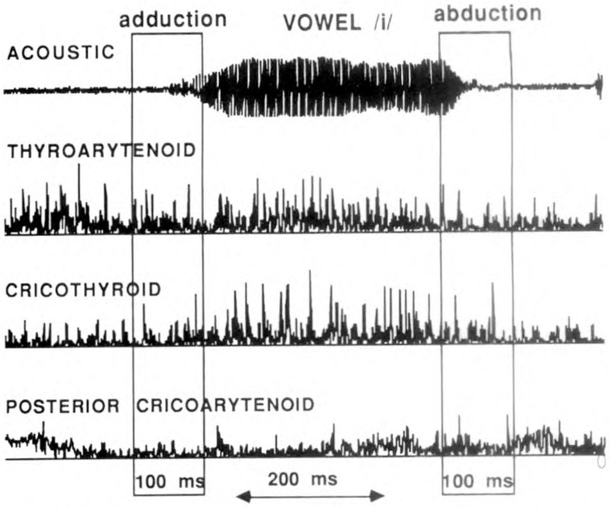

Fig 2. Electromyography recordings from normal speaker, who participated in previous study,[22] to illustrate method used to measure mean muscle activity during vocal fold adduction and abduction during particular speech gestures. Measurement windows included 1) 50-ms intervals before and after phonation onset, totaling 100 ms during vocal fold adduction, and 2) 50-ms intervals before and after phonation offset, totaling 100 ms during vocal fold abduction.

Although the mean values varied from token to token in the right and left TA muscles, a similar pattern was seen on the 2 sides. The mean level of TA activity during vocal fold abduction for the /s/ was always greater than during the vocal fold closure for the vowel /i/. As seen in Fig 3, the 2 TAs and the other 3 muscles increased in activity near the end of /i/ before vocal fold opening for /s/. This was surprising, given the expectation that the TA muscle would be more active for vocal fold adduction for /i/ than for abduction for the /s/. Also in Fig 4, the patterns of activity in the right and left CT muscles differed considerably and varied across tokens, with the level of activity most often being greater for abduction than for adduction. This was also true in the PCA; activity for abduction was greater than for adduction during all but 1 of the 9 syllable productions.

Similarly, the muscle signals from the same subject were measured during repeated production of /ʔi/ (Fig 5). For this syllable type, the muscle patterns varied considerably between repetitions. The mean muscle activities for adduction and for abduction are plotted for 4 repetitions of this syllable in Fig 6. The right and left TA muscles were somewhat similar. However, the mean level of TA muscle activity was greater during adduction on this task, and the same trend was noted in the other 3 muscles. Therefore, the mean level of activity was greater for *abduction* during /si/ in all 5 muscles and greater for *adduction* in the same muscles during /ʔi/. All the muscles, therefore, were active during a change in vocal fold position from the phonatory position, regardless of whether the direction required adduction or abduction. Such findings make conclusions about the bio-

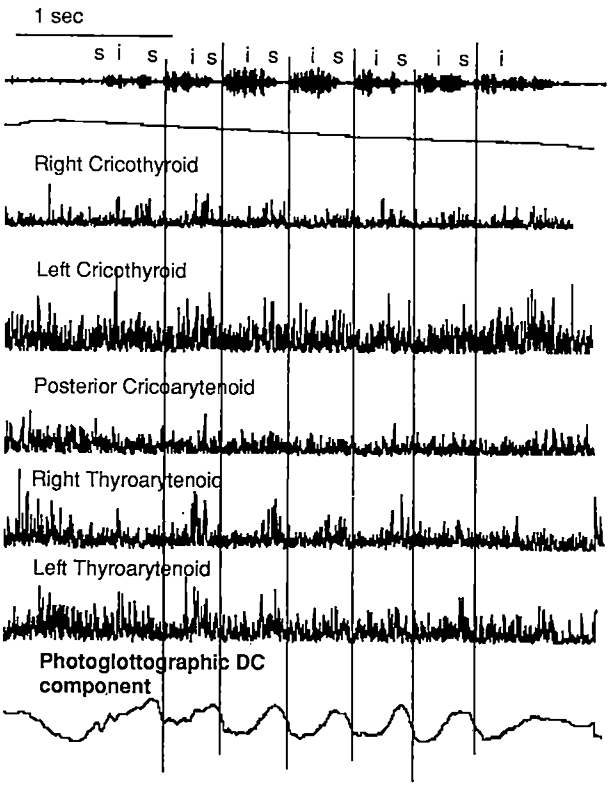

Fig 3. Simultaneous electromyographic recordings from right cricothyroid, left cricothyroid, posterior cricoarytenoid, right thyroarytenoid, and left thyroarytenoid during repetition of syllable /si/ by normal speaker. Consistency in glottal area changes across syllables can be seen in display of photoglottographic signal at bottom. Acoustic speech signal is displayed at top and was used for identifying 100-ms adduction and abduction intervals for measuring mean muscle activity.

mechanical function of each of the laryngeal muscles very difficult and highly gesture-dependent. During speech, the muscles were constantly active, with only slight modulations in muscle tone. These slight variations in tone modulation seem to be adequate for vocal fold adduction or abduction. In addition to these small changes in muscle activity, aerodynamic factors may also contribute to changes in glottal opening during speech. For example, if the subglottal pressure is great, then only a small change in muscle activity may be needed for vocal fold abduction.

To examine the consistency of the muscle activation patterns among normal speakers, 5 normal volunteers, naive to the purpose of the study and without medication,[23] were studied after informed consent. Mean muscle activity was measured during 100-ms intervals, including the 50-ms intervals before and after speech onset or offset, in 4 different speech gestures. These were 1) adduction for /i/, 2) abduction for /s/ after /i/, 3) adduction for /ʔ/ interrupting /i/, and 4) abduction for /i/ following /ʔ/. Hooked wire recordings were made simultaneously from the TA and CT muscles, and all subjects had the PCA muscles recorded by means of a surface hypopharyngeal electrode[24] for which accurate placement was verified by sniff and deep inhalation. Two of the subjects had simultaneous recordings made on the left side of the sternothyroid and thyrohyoid. In all subjects, the signals were band-pass-filtered (100 to 5,000 Hz) and amplified. Digitizing was off-line at 5,000 samples per second, with anti-aliasing filtering at 2,000 Hz. After conversion to microvolts and extraction of the DC shift and noise from rectified signals, the mean level of activity of each of the 5 muscles was measured in microvolts in 100-ms windows during phonation onset and offset (Fig 2).

Between-subject differences in muscle activation patterns for speech were examined. Within each subject, we measured the mean amount of muscle activity of each adduction and abduction across all the repetitions of either /si/ or /ʔi/ within a subject. To determine if a subject's mean muscle activity for abduction differed from that for adduction during syllable productions, the mean amount of muscle activity during abduction for /s/ was subtracted from the mean amount of activity during adduction for /i/. A ratio of the difference in the 2 mean values was then computed by dividing the difference by the mean amount of activity during abduction for /s/. This ratio was then multiplied by 100 to compute the percent increase in activity for adduction for /i/ following /s/ over that during abduction for /s/, in each muscle in each subject. If the TA and PCA muscles were reciprocal, that is, the TA was active for adduction while the PCA was active for abduction, these ratios would all have been positive for the TA and negative for the PCA. Similarly, the percent increase in muscle activity for adduction for /ʔ/ over abduction after /ʔ/ was computed. Here, reciprocal activity should have produced negative results for the PCA and positive results for the TA. The expected patterns between the TA and PCA were not found (Fig 7). None of the subjects had negative results on the PCA on /ʔ/, and 1 subject even had a positive value for the PCA during abduction for /s/. Further, for the TA muscle, 2 subjects had negative results during adduction for /i/, indicating that these speakers had greater TA activity during abduction than during adduction. Therefore, the pattern of muscle activation varied between subjects, and the expected use of the muscles for adduction and abduction varied between tasks.

In summary, the within- and between-subject variability in muscle activation patterns was substantial in normal speakers and often was not in the expected direction. This would significantly impede the ability to determine when a patient has abnormal muscle activation patterns. Certainly, the activity in expected antagonist pairs is not reciprocal. Rather, all of the muscles were constantly active to some degree, with only small modulations in activity occurring. The pattern of modulation differed not only within an individual speaker while saying the same syllable, but also between normal speakers. Further, that all of the laryngeal muscles were active throughout speech gestures suggests that it is the interactions between

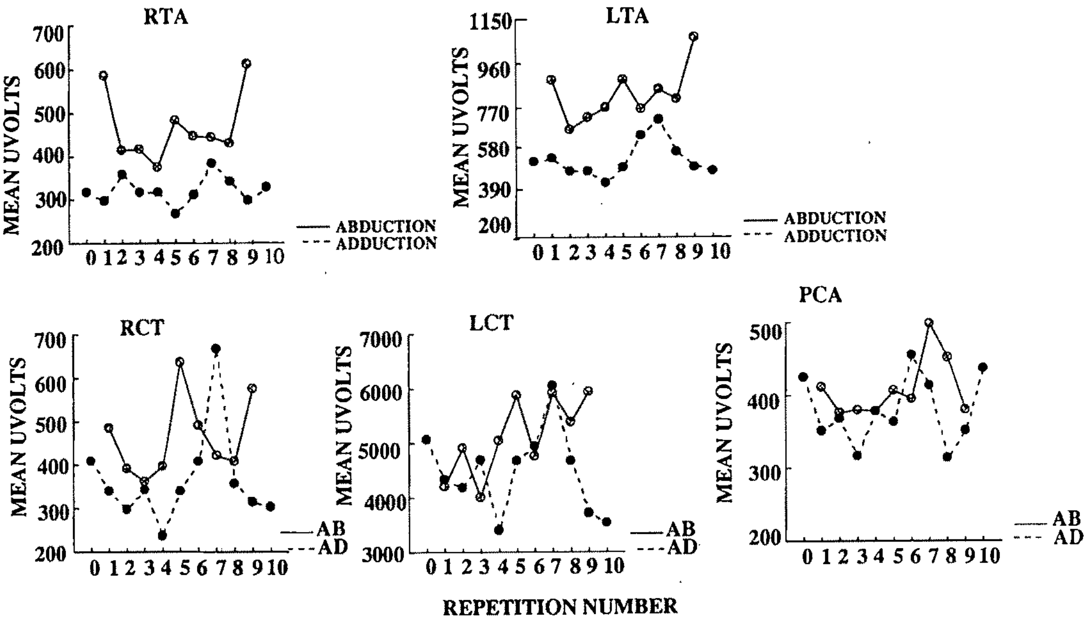

Fig 4. Plot of mean level of muscle activity computed in 100-ms windows, from 50 ms before adduction (AD) or abduction (AB) gesture to 50 ms after AD or AB gesture. Mean values for AD and AB portions of each repetition by subject are plotted for each of 5 muscles studied. Speaker was repeating syllable /si/ 10 times, 6 times on single breath as shown in Fig 3 and during another series of 4 repetitions. DC component of photoglottographic signal tracing on bottom represents changes in glottal area occurring across repetitions. Acoustic speech signal at top was used to identify points of phonation onset and offset for setting averaging windows in each syllable production. RTA — right thyroarytenoid, LTA — left thyroarytenoid, RCT — right cricothyroid, LCT — left cricothyroid, PCA — posterior cricoarytenoid.

many muscles that may play a role in changing vocal fold position, rather than a burst of activity in any 1 muscle. This interactive coordination between all the laryngeal muscles during movement may explain why the activation pattern of individual muscles is highly variable over time within and between subjects.

In conclusion, given the complexities in normal subjects' patterns of muscle activation, it is unlikely that clear-cut criteria can be developed for identifying abnormal patterns of muscle activity.

VARIATION IN MUSCLE ACTION POTENTIAL ONSET LATENCIES DURING PERIPHERAL MAGNETIC STIMULATION OF LARYNGEAL NERVES

Background and Purpose. To determine whether or not a patient will recover from a vocal fold paralysis due to RLN injury often depends on assessing the site and magnitude of the peripheral nerve injury.[16] In clinical neurophysiology, electrical stimulation is used to measure nerve conduction time, with surface electrodes placed at a well-defined site for nerve stimulation. Increased conduction times greater than 3 SD from the normal mean are used to identify a peripheral neuropathy.[25] The laryngeal nerves, however, are difficult to access by means of surface electrical stimulation because of their depth and variation in course. Although intraoperative stimulation is possible, few have reported the use of electrical stimulation using either percutaneous needles or surface stimulation. Thumfart[3] reported that stimulation intensities (50 to 100 V) on the surface of the hyoid cornu just in front of the sternocleidomastoid muscle could induce responses in the CT and the TA muscles to demonstrate intact function in the SLN and RLN, respectively. He suggested that a lack of response was associated with neuropathology in either nerve; however, because of the depth of the laryngeal nerves and variations in the degree of depth of the nerves between individuals depending on fatty tissue, etc, this criterion for identifying neuropathology may need to be guarded. For surface stimulation of a nerve, electrical stimulation is usually required at high intensities that can be quite painful, because the stimulus is high enough to activate pain fibers.[26] More recently, Thumfart et al[12] have suggested that magnetic stimulation offers an easy, noninvasive possibility for measuring the nerve conduction time in order to identify a peripheral neuropathy. For limb musculature, this technique can be used to stimulate the nerve roots at the spinal cord, producing conduction times comparable to those measured by F waves by means of electrical stimulation.[27] The F wave is due to motor neuron activation by an antidromic nerve volley and thus estimates nerve conduction time.[27] For cranial nerves, however, the site of magnetic stimulation is less clear, with the possibility that the stimulation may produce a response in the peripheral nerve, in the nerves as they emerge from the skull, or perhaps in the brain stem.[12,28-32]

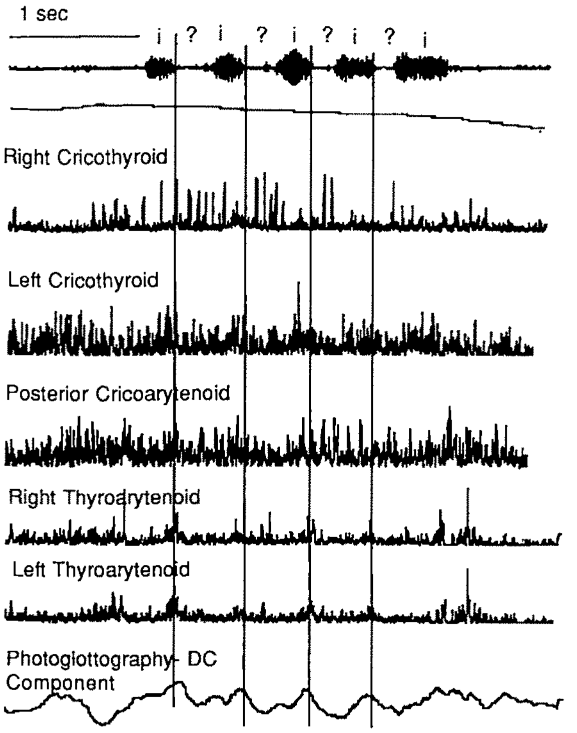

Fig 5. Simultaneous electromyographic recordings from right cricothyroid, left cricothyroid, posterior cricoarytenoid, right thyroarytenoid, and left thyroarytenoid during repetition of syllable /?i/ (glottal stop and vowel) produced by normal speaker. Consistency in glottal area changes across syllables can be seen in display of photoglottographic signal at bottom. Acoustic speech signal is displayed at top and was used for identifying 100-ms adduction and abduction intervals for measuring mean muscle activity.

Thumfart et al[12] reported on the use of magnetic stimulation to elicit a muscle action potential in the TA muscle to determine RLN response latencies. They placed the stimulator "approximately 2 cm behind and 6 cm above the external auditory canal for stimulation of the nerves at their cisternal part"[12(p631)] in 52 healthy subjects. The mean response in the TA muscle was 5 ms, with an SD of 0.7 ms and a range from 4 to 6.6 ms. They also reported that magnetic stimulation on the neck produced a muscle action potential that was not statistically different from that obtained electrically, around 6 to 8 ms in the TA muscle. It is difficult to understand how a more central stimulation point, such as the cisternal location, would produce a TA muscle response at 5 ms, which would be earlier than responses elicited by peripheral nerve stimulation occurring between 6 and 8 ms. These findings suggest that the sites of stimulation might not be well understood.

Similar difficulties have been found with the ability to distinguish the exact site of stimulation for the facial nerve. Rimpilainen et al[30] identified 3 groups of responses in the nasolabial folds with transcranial magnetic stimulation and suggested that the pathway may be activated at several sites simultaneously: the intracranial part of the trigeminal nerve producing sensorimotor responses such as R1 and R2; the brain stem; or the facial nerve itself. They and others have compared transcranial magnetic stimulation responses with intraoperative electrical stimulation of the facial nerve at the root exit zone and more distally at the entrance to the petrous bone.[33-35] Different conclusions have been reached; 1 study concluded that the root exit zone was the location of stimulation,[35] while 2 others concluded that stimulation of the nerve was within the internal auditory meatus.[33,34] In contrast, an intraoperative study aimed at locating the site of stimulation of the trigeminal nerve demonstrated that the excitation site was variable among individuals, located between 1.6 and 6.5 cm distal to the root exit zone.[36]

Our purpose was to evaluate the consistency of TA muscle response latencies to peripheral magnetic stimulation results in normal speakers. If consistent response latencies occurred between individuals, this technique might be used to determine nerve conduction time for the RLN. To measure nerve conduction time, 1 criterion for diagnosing peripheral nerve injury, the stimulation site must be consistent for one to determine if the response latency is prolonged.[27] Therefore, we attempted to control the stimulation site by using a well-defined method of stimulus presentation.

Methods. Six normal subjects, 2 female and 4 male, had normal symmetric laryngeal movement on fiberoptic examination during speech, laughter, and whistling and no history of neurologic or laryngeal disease. After informed consent, bipolar hooked wire electrodes were placed in the TA muscles on both sides by means of percutaneous insertion.[37] Accurate placement was determined with verification gestures such as cough, throat clearing, phonation, and the Valsalva gesture. The EMG signals were amplified and filtered with a pass-band of 100 to 5,000 Hz.

The subjects were placed in a supine position with the neck extended. We selected the Cadwell MES-10 with a Cadwell Round Coil having a 9.5-cm outside diameter and 2.2-T maximum output. Because of the depth of the course of the laryngeal nerves and individual differences in neck size, the round coil, which can stimulate at greater depth, was used, although it had a less focal stimulation region than the figure eight coil.[38] We stimulated over the neck in the region of both the SLN and the RLN. The middle edge of the stimulator was held at an angle of the jaw and angled at 135° from the neck close to the mandible (the J0 position). The current direction in the coil was in the opposite direction from the RLN, because the induced current is in the opposite direction from the current in the coil[39] (Fig 8). A metal plate containing mu-metal was used to cover the laryngeal area, the electrodes, and the electrode connector box, to reduce stimulus artifact. We stimulated on the right and left sides. An electrocardiogram signal was monitored for bradycardia, and the stimu-

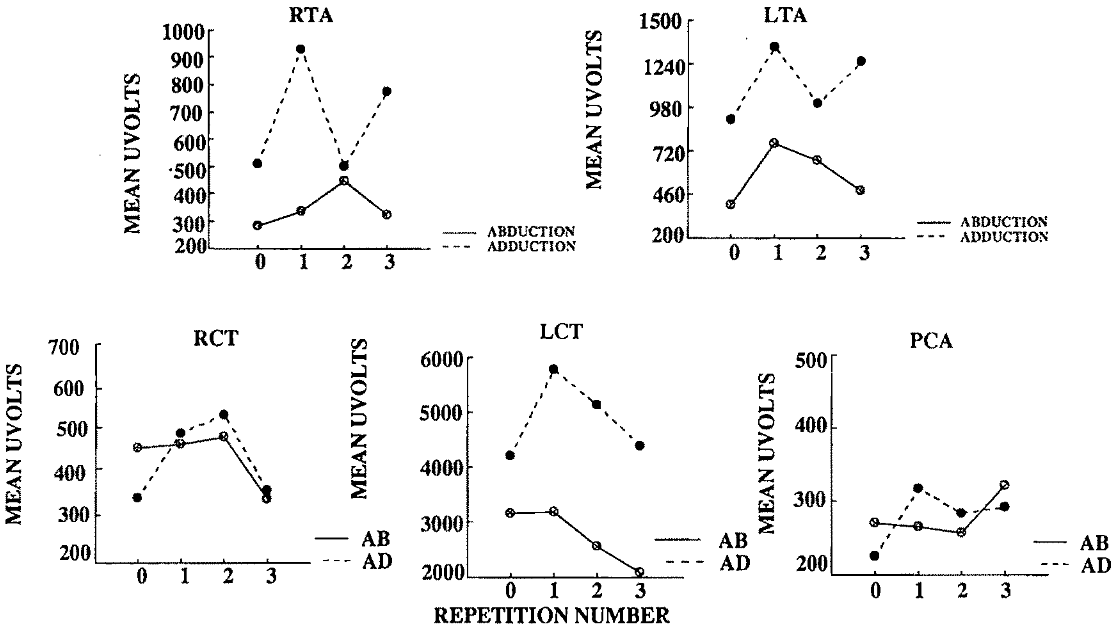

Fig 6. Plot of mean level of muscle activity computed in 100-ms windows from 50 ms before adduction or abduction gesture to 50 ms after adduction or abduction gesture. Mean values for adduction and abduction portions of each repetition by subject are plotted for each of 5 muscles studied. Speaker was repeating syllable /ʔi/ 4 times on single breath as shown in Fig 5. DC component of photoglottographic signal tracing on bottom represents changes in glottal area occurring across repetitions. Acoustic speech signal at top was used to identify points of phonation onset and offset for setting averaging windows in each syllable production.

lation rate was random, with an interval of at least 6 seconds between stimuli.

The response of the ipsilateral TA muscle was examined on a storage oscilloscope over 50 ms. The output level of the magnetic stimulator was increased gradually in 5% to 10% intervals starting at 40% of maximum output. The Cadwell was triggered from a Master 8 stimulator, and the trigger and the EMG signals were simultaneously recorded on a TEAC instrumentation tape recorder. Once a response was observed in the ipsilateral TA, the stimulus output was increased until a consistent supramaximal response was obtained for at least 6 stimuli, usually around 80% of maximal output. The same procedure was used when stimulating at 4 locations, J0 and at 1, 2, and 3 cm above J0, on both sides. The trigger stimuli and the EMG recordings were digitized at 5,000 samples per second. The 6 supramaximal responses, which were all similar, were averaged for each stimulus location in each subject.

Results. The mean right TA responses to supramaximal stimulation at J0 on the right for the 6 subjects are presented in Fig 9. The initial deflection off the baseline in each tracing is the stimulus artifact. In many cases the stimulus artifact persisted for several milliseconds and may have obscured the onset of the muscle action potential. Therefore, sometimes the onset of the M wave is difficult to discern. Usually a return of the signal to baseline is required before the muscle response can be identified. The response onsets are marked for each subject and vary: 2.5 and 4.3 ms in M4, 5.7 ms in M3, 4.3 ms in F2, 6.4 ms in M2, 5.7 ms in M1, and 13.6 ms in F1. It is possible that the late response in F1 is either a long latency response due either to antidromic stimulation producing an F wave, or a very early R1 response due to stimulation of the internal branch of the SLN.[40] If a clinician was confronted with only 1 of the subjects' responses at a time, different muscle onset latencies would be identified. It is likely that these waveforms represent several different sites of stimulation. The earlier response at 2.5 ms in M4 might be due to stimulation of the RLN as it enters the larynx, rather than the proximal descending portion of the RLN. Another possibility is that the electrode is recording proximate CT responses to external SLN stimulation.

Next, the consistency between responses to stimulation on the right and left sides and with a change in stimulator placement was evaluated. The averages of 6 consistent TA responses at 2 stimulator locations, at J0 and 3 cm up from J0 on the left side, are presented in the first 4 subjects in Fig 10. After the stimulus artifact, the first deflection off the baseline should be the muscle response to RLN stimulation. A vertical dashed line is drawn at the first deflection after return to baseline for each response pair in each subject. These muscle response times vary widely among the 4 subjects, from 4.7 to 8.6 ms, and differ from the response times measured on the right side. Overall, however, the response waveform varies very little in shape with changes in stimulator placement over a distance of 3 cm. The major differences with changes in stimulator placement were in response amplitude. This suggests that as stimulator placement is changed,

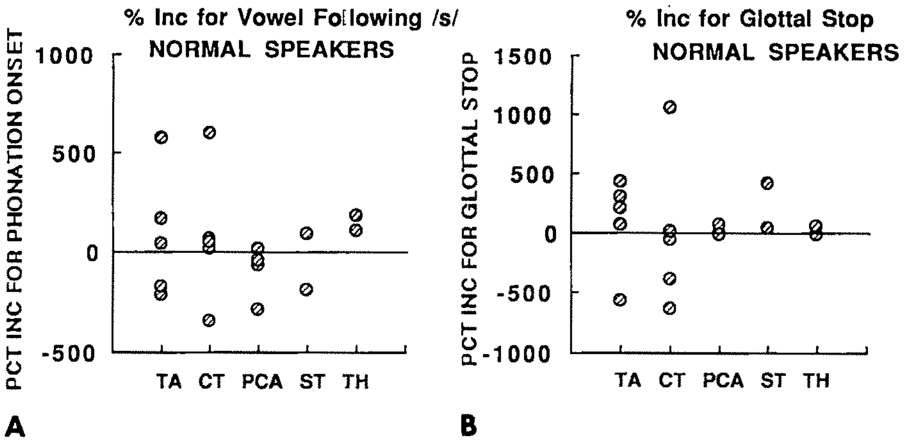

Fig 7. TA — thyroarytenoid, CT — cricothyroid, PCA — posterior cricoarytenoid, ST — sternohyoid, TH — thyrohyoid. A) Mean amount of muscle activity for /s/ abduction was subtracted from mean amount of activity during adduction for /i/ for each subject. Ratio of this difference in 2 mean values was then computed by dividing difference between 2 mean values by mean amount of activity for abduction for /s/. This ratio was then multiplied by 100 to compute percent increase (PCT INC) in activity for adduction for vowel following /s/ over that for abduction for /s/ in each muscle in each subject. B) Mean amount of muscle activity for /i/ abduction was subtracted from mean amount of activity during adduction for /ʔ/ for each subject. Ratio of this difference in 2 mean values was then computed by dividing difference between 2 mean values by mean amount of activity for abduction for /i/. This ratio was then multiplied by 100 to compute percent increase in activity for adduction for /ʔ/ over that for abduction for /i/ in each muscle in each subject.

the site of stimulation does not vary within a subject, but rather, the distance of the stimulator from the stimulation site. However, whether J0 or 3 cm above it was the site of greatest response also varied between the subjects. The response was greater at J3 in F1 and F2, while it was greater at J0 in M1 and M2. Thus, normal subjects varied considerably in their response latencies and the site of stimulator placement that had the greatest response.

Finally, we expected that the TA muscle responses to RLN stimulation would be earlier on the right than on the left within subjects. By examining the responses of F1, F2, M1, and M2 at J0 in Figs 9 and 10, no such consistent differences in muscle response latencies could be found between the 2 sides. These data suggest that responses to stimulation at this position on the neck were not from a single stimulation site across subjects or on the right and left sides. Therefore, this was not a reliable method for measuring nerve conduction time in the RLN and might not be a useful method for differentiating between those with a peripheral neuropathy and those with normal nerve function.

DISCUSSION

For assessment procedures to be useful in diagnosing patient abnormalities, the normal range of function must be well defined for one to distinguish patients with abnormalities. In this paper, the follow-

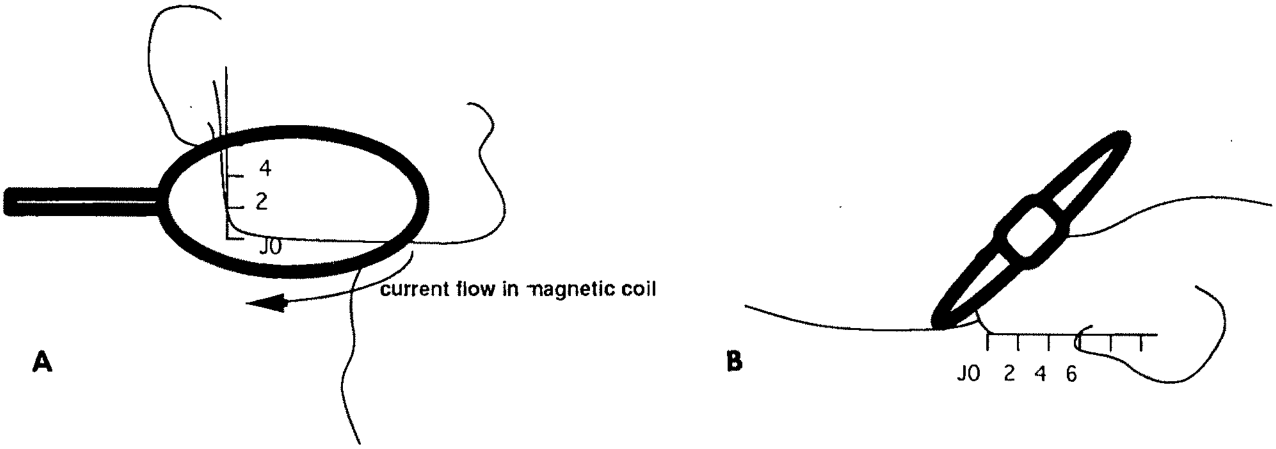

Fig 8. Schematic representation of placement of magnetic stimulator for peripheral laryngeal nerve stimulation. A) Lateral view. B) Posterior view.

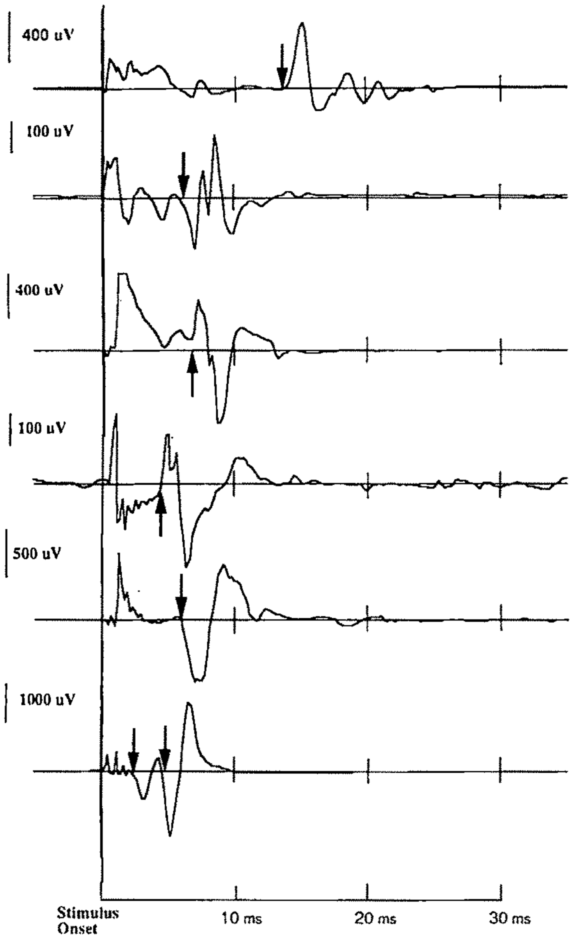

Fig 9. Mean of 6 right thyroarytenoid responses to supramaximal stimulation at J0 (jaw angle) on right for 6 normal subjects (2 women and 4 men). Initial deflection off baseline in each tracing is stimulus artifact immediately following stimulus onset. Arrows are drawn to identify onset of muscle action potential response to magnetic stimulation.

ing difficulties in the reliable use of EMG of the laryngeal muscles were discussed.

1. Difficulties in recording selectively from 1 of the laryngeal muscles without picking up signals from adjacent muscles when not using bipolar needle electrodes.

2. Difficulties in positioning electrodes in the superior anterior portion of the TA muscle, where the muscle is not adjacent to the CT muscle.

3. The possibility of physiologically distinct compartments within the laryngeal muscles.

4. The variation in firing patterns between motor units within the same muscle.

5. The variation in the vocal fold movements and postures used by normal subjects in producing the same speech sounds.

Second, we examined the regularity of muscle activation patterns within and between normal speakers and found

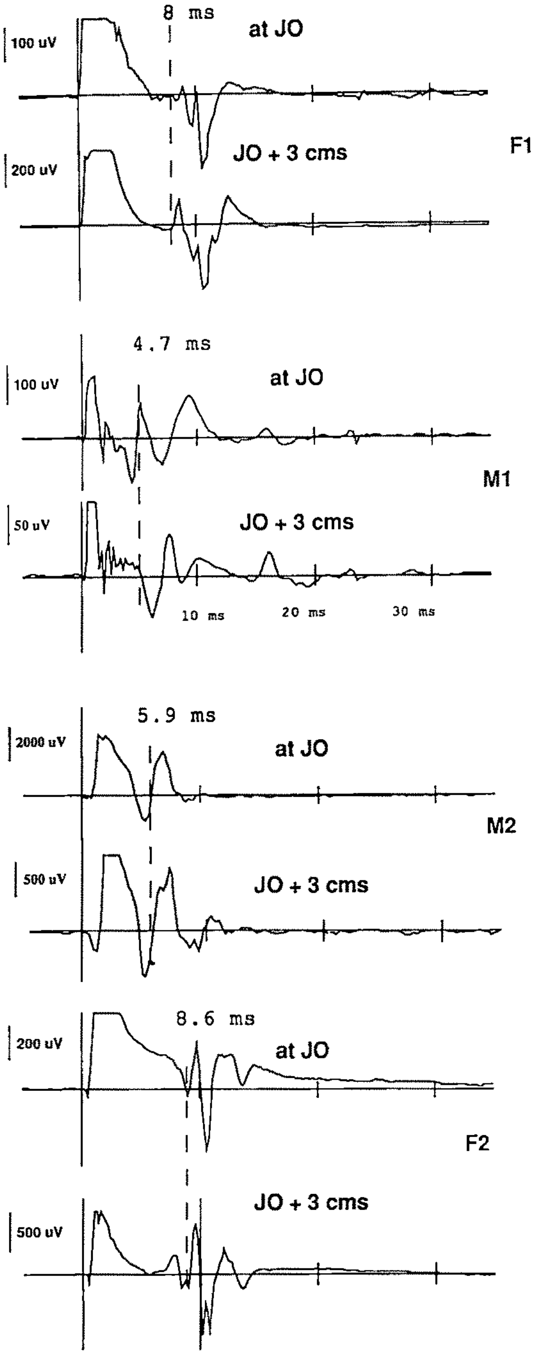

Fig 10. Consistency between ipsilateral responses to stimulation on left side and with change in stimulator placement. Averages of 6 supramaximal thyroarytenoid responses at 2 stimulator locations, at J0 and 3 cm up from J0 on left side, are shown for first 4 subjects shown in Fig 9. Vertical dashed line is drawn at first deflection, after return to baseline, for each response pair in each subject, which should be muscle response to recurrent laryngeal nerve stimulation.

1. During repetitions of 2 different speech syllables, a normal speaker had increased activity in all

muscles recorded during the *abduction* portion in 1 syllable, and increased activity in the same muscles during the *adduction* portion of another syllable.

2. During the same speech gestures the difference in muscle activity between adduction and abduction varied across subjects on each muscle; this finding indicates that the same muscle was used differently for vocal fold movement by normal subjects.

3. Muscles thought to have 1 specific function, either for vocal fold adduction or abduction, were not active during just 1 gesture in normal speakers.

Third, when using peripheral magnetic stimulation to test nerve conduction time in normal speakers, the high degree of variability in muscle response latencies and patterns suggested that even when an external placement of the stimulator is consistent, the stimulation site(s) producing muscle response(s) may vary. By placing the stimulator on the neck below the mandible, we had hoped to stimulate both the SLN and RLN. However, difficulties accessing only the TA muscles with the percutaneous approach, and the poor specificity of the bipolar hooked wire electrodes, may have compromised the results. Some of the earlier responses may have been from the electrode's recording CT muscle responses to SLN stimulation. Because of the shorter length of the SLN, CT responses may occur around 3 to 4 ms. These might explain some of the earlier responses that occurred in M1 and M4 in Fig 9 and in M1 in Fig 10. Improved methods are needed for accessing the TA and the other laryngeal muscles. Although Thumfart[3] and others[41] have used the peroral approach, considerable anesthesia is needed, and subjects cannot tolerate the electrodes for more than a few minutes. New methods are needed that allow for selective recordings that can be maintained over time during speech, other movement gestures, and magnetic stimulation.

Electrode placement problems may have also affected the muscle activation patterns presented in this paper for the TA and PCA muscles. Although verification gestures were used, because the percutaneous approach to electrode placement was used, some TA recordings may have included activity from the LCA and CT muscles. Further, the PCA recording was made with a surface electrode. Again, although verification gestures were used, and this electrode is accurate for recording PCA activity during vocal fold abduction,[24] either the electrode may have moved during testing or it may have also recorded from that portion of the interarytenoid muscle close to the PCA. Therefore, caution must be used in interpreting these results.

Our expectations of reciprocal activation patterns during vocal fold adduction and abduction during speech were based on previous reports in the literature in which investigators averaged many repetitions of a speech item. Such averages are useful for identifying an average level of a muscle, but are not useful for determining how a muscle might vary in its activity and to what degree the muscles interact during movement. The data presented here suggested a very different model of laryngeal muscle action during speech. They suggest that for speech the muscles had tonic rather than phasic activity patterns. That the levels of activity were very low and varied only slightly suggests that the interactions between the muscles, along with aerodynamic factors, may play a role in vocal fold movement for phonation onset and offset during speech.

In this study we used speech tasks in our attempts to identify common patterns of muscle activity in normal subjects. Another alternative might be to use nonspeech gestures such as swallowing, coughing, and the Valsalva maneuver. In our experience and that of others,[4,6] these gestures are highly variable in subjects as well. Movement artifacts interfere with laryngeal EMG recordings during swallowing and coughing. The Valsalva gesture is useful for identifying vocal fold adduction, but requires considerable amounts of training to be produced even by normal subjects. We expected that because speech is an overlearned motor skill used daily, it would be easy for subjects to perform consistently without training.

Our results at the present time suggest that more technical development is needed before laryngeal EMG and magnetic stimulation can be used reliably for evaluating patients with laryngeal motor control disorders. Our EMG findings are only preliminary. The speech gestures studied were quite complex, and improved reliability might have been found with simple gestures such as phonation. Nonetheless, these results suggest that laryngeal biomechanics are far more complex than simple anatomic models would suggest. Rather than there being reciprocal actions of adductor and abductor muscles, none of the laryngeal muscles seem to work in isolation. Interactive tonic modulations among many muscles seem required to achieve precise control of vocal fold shape and tension for voice. Future advances in neurolaryngology will depend on increasing our understanding of laryngeal biomechanics and laryngeal neural control. A fruitful approach would be to start with a few simple, well-defined gestures for study. Such gestures might become the basis for reliable diagnostic procedures for evaluating laryngeal motor control disorders.

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MICROLARYNGOSCOPIC SURGERY FOR T1 GLOTTIC LESIONS: A COST-EFFECTIVE OPTION

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Excision of squamous cell carcinoma of the vocal cord using microlaryngoscopy (ML) with or without laser is a cost-effective treatment option in highly selected patients. A retrospective review of 50 cases of invasive and microinvasive squamous cell carcinoma demonstrated a failure rate of 8% (4 patients), all of whom were successfully salvaged by hemilaryngectomy (1 patient) or irradiation (3 patients). Other treatment options for T1 glottic lesions include hemilaryngectomy or radiotherapy, yielding similar cure rates of 95% and 67% to 93%, respectively. Investigation of the potential savings by extrapolating 1992 health care costs into the treatment options for T1 glottic carcinoma resulted in costs of $12,956 per patient for ML, while hemilaryngectomy averaged $35,616 per patient and radiotherapy netted costs of $32,588 per patient. Extrapolating these costs to a theoretic group of 100 patients, ML provided savings of 2.4 million dollars when compared to the other treatment modalities. In selected patients, ML for T1 glottic cancer provides an effective therapeutic modality suited to our cost-conscious environment.

KEY WORDS — cost-effectiveness, glottic carcinoma, laser excision, microlaryngoscopy.

INTRODUCTION

Current standard treatment options for T1 glottic lesions include microlaryngoscopy (ML) with excision of the lesion with or without use of a laser, vertical partial laryngeal surgery, and irradiation. All three modalities have demonstrated primary cure rates of greater than 85%. Secondary salvage attempts have demonstrated total cure rates of greater than 90%[1-4] with all three treatment modalities.

However, with increased insurance constraints, physicians must now consider treatment costs along with medical decision-making to provide cost-effective treatment.

METHODS

A retrospective review of charts of patients treated in the Department of Otolaryngology, University of Pittsburgh School of Medicine, was performed to determine the outcome in patients who underwent ML plus excision with or without laser as primary therapy for T1 glottic lesions with invasive or microinvasive squamous cell carcinoma. If the patient met the inclusion criteria, the following data were obtained: age, sex, site, stage, diagnosis, date, number of ML procedures before the primary procedure, number of operative procedures after the primary oncologic procedure, smoking history, smoking cessation, pathology, status, and follow-up.

COST ANALYSIS

Cost analysis was performed for each treatment modality and hospitalization by averaging 10 hospital bills and related service bills for patients undergoing the procedure and/or surgery for that particular treatment modality in 1992. Therefore, the average cost of each procedure includes hospital bills, anesthesia, anesthesiologist, radiologist, radiology, ancillary departments, radiation oncology, radiation oncologist, surgery, and surgeon's fees, if applicable.

Once all data were collated it was possible to extrapolate our data into a theoretic group of 100 patients undergoing ML plus excision with or without laser so that cost-benefit savings could be analyzed. From a review of the literature we obtained the primary failure rate for the other two treatment modalities for T1 glottic carcinoma. Finally, a cost analysis among all three groups of patients was performed.

RESULTS

Demographics. Fifty patients underwent ML with excision of squamous cell carcinoma with or without laser between 1978 and 1990. The male to female ratio was 3 to 1 and the average age was 63 years (range 22 to 82 years). All tumors were American Joint Committee on Cancer[5] stage T1N0.

Presentation. The vocal cord lesions were classified as anterior, middle, or posterior lesions on the basis of dividing the vocal cords into thirds. No patient had involvement of the anterior commissure. Anterior and midcord lesions were predominant,

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TABLE 1. COSTS OF TREATMENT FOR GLOTTIC CARCINOMA

Type of Treatment	*Total Costs**
Microlaryngoscopy plus excision with or without laser (1 procedure)	$6,478
Microlaryngoscopy plus excision with or without laser (2 procedures)	$12,956
Radiotherapy	$27,000
Hemilaryngectomy	$35,616
Total laryngectomy	$25,649
Laryngoscopy	$5,588

*Costs include hospital, surgeon, radiology, radiation oncology, ancillary services, and both technical and professional components, where applicable (1992 figures).

TABLE 2. COSTS FOR THEORETIC GROUP OF 100 PATIENTS UNDERGOING MICROLARYNGOSCOPY WITH EXCISION FOR T1 GLOTTIC CARCINOMA

ML (100 patients) = ($6,478 × 2) × 100 patients = $1.3 million.
Eight patients would fail this regimen and need further treatment.
Two patients would have HL for salvage and 6 patients would undergo XRT. Therefore,
HL (2 failures) = $35,616 × 2 patients = $71,232
and
XRT (6 failures) = $27,000 × 6 patients = $162,000.
Finally, total costs for the entire ML patient group would be
C (total) = ML + HL + XRT or $1.5 million.

ML — microlaryngoscopy, HL — hemilaryngectomy, XRT — radiotherapy, C — cost.

with 24 patients (48%) in each group, while posterior lesions comprised only 4% of the lesions. Ninety-six percent of patients presented with symptoms of hoarseness having an average duration of 7.4 months (range 1 to 36 months). Most patients also presented with a long smoking history: 30 patients (60%) had a greater than 60–pack-year history, 16 patients (32%) had a less than 60–pack-year history, and only 2 patients (4%) had no previous smoking history. Smoking history was not available on an additional 2 patients (4%).

Surgery. All patients treated by ML and endoscopic excision were managed on an outpatient basis. The patients were admitted to the Eye and Ear Hospital early on the morning of surgery and discharged by early afternoon, thus obviating the cost of an inpatient day.

Only 4 patients in our study group had endoscopic excision without use of a laser. The remaining 46 patients (92%) had surgical removal of the lesion with a laser. Pathology revealed that 18 patients (36%) had carcinoma in situ with microinvasion, and the additional 32 patients (64%) demonstrated invasive squamous cell carcinoma on final pathology.

Outcome. Forty-six patients (92%) were cured by primary ML with excision of the lesion. The average follow-up on this group of patients was 48 months, with a range of 24 to 157 months. Patients cured with primary ML therapy required an average of 2 procedures, with a range of 1 to 6 ML procedures. Smoking cessation counseling was presented to all patients as part of the treatment plan. Thirty-eight (76%) of all of the patients were successful in not smoking after diagnosis.

Four patients (8%) in our study failed ML treatment. Of the 4 who failed, 3 patients had anterior cord lesions and 1 patient had a midcord lesion. Three of the 4 patients requiring secondary therapy stopped smoking after the primary surgery, while the other patient continued to smoke. Subsequently, all 4 patients were salvaged and retained their voice: 1 patient (2%) by hemilaryngectomy and 3 patients (6%) by irradiation.

Cost Analysis. Using treatment costs described in Methods, we determined the costs for each type of procedure (Table 1). Of interest, the higher dollar cost of the hemilaryngectomy treatment is due to the longer hospitalization and additional ancillary services required for patients undergoing hemilaryngectomy. Extrapolating data from this review into a theoretic group of 100 patients revealed that 92 patients would be cured by ML with 2 procedures (the average number of ML procedures needed for cure). The theoretic 8 primary treatment failures would be treated with hemilaryngectomy (2) or irradiation (6). Therefore, the total cost for 100 patients receiving primary treatment with ML and subsequent salvage therapy would be 1.5 million dollars (Table 2).

Using our own data to analyze costs for a group of patients undergoing hemilaryngectomy for T1 glottic carcinoma, we would expect a primary cure rate of 96%.[3] Four failures would be treated by either a total laryngectomy or irradiation. Calculations for a theoretic group treated with hemilaryngectomy yield a total cost of 3.6 million dollars (Table 3).

Finally, historical data can also be applied to a theoretic group of patients undergoing primary radiotherapy. Cure rates in the literature range from 67%[6] to 93%[7]; therefore, we picked a cure rate of 80% (the average of both ends of the range) for our theoretic irradiation group. Therefore, the total cost for this group is 3.9 million dollars (Table 4).

It is then possible to compare all three theoretic groups for total cost savings per 100 patients. The ML plus excision group would cost only 1.5 million dollars, whereas the hemilaryngectomy group would cost 3.6 million dollars or 2.1 million additional health care dollars. Irradiation in a group of patients

TABLE 3. COSTS FOR THEORETIC GROUP OF 100 PATIENTS UNDERGOING HEMILARYNGECTOMY FOR T1 GLOTTIC CARCINOMA

HL (100 patients) = \$35,616 × 100 patients = \$3.5 million.

Assuming of the 4 failures, 2 would undergo TL while 2 would undergo XRT for salvage, then

TL (2 failures) = \$25,649 × 2 patients = \$51,298

and

XRT (2 failures) = \$27,000 × 2 patients = \$54,000.

Therefore, total costs for entire HL patient group would be

C (total) = HL + TL + XRT or \$3.6 million.

HL — hemilaryngectomy, TL — total laryngectomy, XRT — radiotherapy, C — cost.

TABLE 4. COSTS FOR THEORETIC GROUP OF 100 PATIENTS UNDERGOING RADIOTHERAPY FOR T1 GLOTTIC CARCINOMA

Patients undergoing XRT would require diagnostic laryngoscopy prior to therapy, which therefore would be included in costs of XRT.

XRT (100 patients) = (\$27,000 + 5,588) × 100 patients = \$3.3 million.

Additionally, 20 patients would fail treatment and require secondary surgical procedure for cure. Arbitrarily, 14 patients (70%) would undergo HL and other 6 patients (30%) would undergo TL.

HL (14 failures) = \$35,616 × 14 patients = \$498,624

and

TL (6 failures) = \$25,649 × 6 patients = \$153,894.

Total costs for entire XRT patient group would be

C (total) = XRT + HL + TL or \$3.9 million.

XRT — radiotherapy, HL — hemilaryngectomy, TL — total laryngectomy, C — cost.

would net costs of 3.9 million dollars, or 2.4 million dollars in additional costs for T1 glottic patients.

DISCUSSION

Our study is consistent with data presented by Blakeslee et al in 1984.[8] Health care is under increased scrutiny in both the government and public sectors. It has become increasingly more popular to manage health care by managing health care dollars. While we do not advocate treatment options be based solely on costs, it is increasingly obvious that cost is an important issue if the outcome of therapy is equal. In small vocal cord lesions, ML plus excision provides results similar to those with open conservation laryngeal surgery or radiotherapy, yet the comparative costs involved in treating a theoretic group of 100 patients demonstrate a potential for savings.

Our analysis has considered only the dollar amounts expended on the actual patient care aspects of these treatment programs. A cost that is difficult if not impossible to ascertain, but is extremely important in the consideration of this matter, is the amount of money lost to the overall economic system in the form of lost workdays for the patient during either radiation treatment or open neck surgery. Throughout the world, radiotherapy remains the standard of care for early cancer of the vocal cord. One of the reasons for this is that it has been thought to be very cost-effective in keeping patients out of the hospital. We believe it is clear that the economic impact of patients not being able to work during a prolonged course of radiotherapy has a major impact both on their income and on what has to be paid out by employers as unemployment compensation. The cost to the employers must have a formidable economic impact.

We conclude that ML with excision is an effective, cost-conscious management option for properly selected patients with early vocal cord cancer.

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Ann Otol Rhinol Laryngol 103:1994

TREATMENT OF DYSFUNCTION OF THE CRICOPHARYNGEAL MUSCLE WITH BOTULINUM A TOXIN: INTRODUCTION OF A NEW, NONINVASIVE METHOD

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Botulinum toxin is known as a relatively safe and efficacious agent for the treatment of various neurologic and ophthalmologic disorders. Since dysphagia and deglutition problems combined with aspiration are often caused by spasticity, hypertonus, or delayed relaxation of the upper esophageal sphincter (UES), conventional treatment including lateral cricopharyngotomy was replaced by localized injections of botulinum toxin into the cricopharyngeal muscle (CM) in a series of 7 patients. The study comprised patients with slight dysphagia caused by isolated hypertonus of the UES, as well as patients with severe deglutition disorders, complete inability to swallow, and aspiration problems. Preoperative diagnostic evaluation included careful history-taking, physical examination, cineradiography, and esophageal manometry to exclude other causes of dysphagia. For precise localization, injections were performed under general anesthesia after location of the CM by direct esophagoscopy and electromyographic guidance. Injections were administered into the dorsomedial part and on both sides into the ventrolateral parts of the muscle. Depending on the severity of symptoms and the intraluminal pressure of the UES, the dose varied between 80 and 120 units (botulinum toxin A from Dysport). The treatment outcome was evaluated by a disability rating score: patients' complaints were scored by subjective and objective parameters before and after injection. All but 2 patients experienced complete relief or marked improvement of their complaints. There were no severe side effects or postoperative complications. Local botulinum toxin injection proved to be an effective alternative treatment to invasive procedures for patients with isolated dysfunction of the UES, and also for patients with more complex deglutition problems combined with aspiration.

KEY WORDS — botulinum toxin, cricopharyngeal muscle, dysphagia.

INTRODUCTION

Botulinum toxin type A, one of the most dangerous biologic toxins, has been found to be of considerable therapeutic value in the treatment of a variety of ophthalmologic and neurologic disorders.[1-3]

In the field of otorhinolaryngology, botulinum toxin has proven to be a relatively safe and efficacious treatment of such facial nerve disorders as synkinesias or hemifacial spasm, laryngeal dystonia, oromandibular dystonia, and spasmodic torticollis.[4-11] Botulinum exotoxin is a large protein molecule. Following intramuscular injection it binds rapidly and strongly to presynaptic cholinergic nerve terminals and impairs the release of acetylcholine at the neuromuscular junction,[12] producing a dose-related weakness of the affected muscle ("chemical denervation"). The clinical effect of botulinum toxin is temporary, lasting up to 6 months.

The goal of our study was to abolish abnormal innervation of the upper esophageal sphincter (UES) and to relax the muscle by chemical denervation. In a series of 7 patients with deglutition problems we replaced conventional treatment, including lateral cricopharyngotomy and laser dissection of the UES, with localized intramuscular injections of botulinum toxin.

PATIENTS AND METHODS

Patients with pathologically increased intraluminal pressure, hypertonus, or hypertrophy of the UES and patients with more complex deglutition and aspiration problems following cerebrovascular accident or resection of endocranial, oropharyngeal, and laryngeal tumors were included. None of the patients responded to prior medication or deglutition training.

In order to first exclude other causes of dysphagia[13] such as globus syndrome, esophageal scars, and cancer, a careful history-taking, including information about duration and frequency of complaints, and an exact description of swallowing problems were mandatory. For analyzing proper function, a physical examination of the motion of the larynx by transnasal fiberendoscopic laryngoscopy and additional swallowing of methylene blue solution was performed. Further investigations included esophageal manometry to measure the intraluminal pressure and coordination and degree of relaxation of the UES. High-speed cineradiography revealed aspiration or nasal regurgitation and localized the area of dyscoordination

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TABLE 1. SUMMARY OF PATIENT DATA

Pt No.	*Age*	*Sex*	*Basic Disease*	*Feeding Tube*	*Nature of Swallowing Disorder*	*Aspiration*	*Pneumonia*	*Behavior of Upper Esophageal Sphincter*	*Injection (mL)*
1	29	M	Stroke, palsies of nerves VI, X, and XII	+	Central neurologic disorder with complex dysfunction	+	+	Missing relaxation	0.4; 0.8
2	52	M	Supraglottic carcinoma, partial laryngectomy	–	Dysphagia for solid and especially liquid food	+	–	Delayed relaxation	0.4
3	47	F	Oropharynx carcinoma, lower jaw resection	–	Dysfunction of pharyngeal and pharyngoesophageal phases	+	–	Missing relaxation	0.4; 0.4
4*	71	M	Acoustic neurinoma, palsies of nerves IX and X, tracheostomy	+	Central neurologic disorder with complex dysfunction	+	+	Manometry not tolerated	0.4
5	55	M	Stroke, tetraplegia, tracheostomy	+	Central neurologic disorder with complex dysfunction	+	–	Hypertonus and delayed relaxation	0.4
6*	45	M	Reflux disease, brachyesophagus	–	Dysfunction of pharyngoesophageal phase	–	–	Hypertonus and hypertrophy	0.4
7	62	M	Oropharynx carcinoma, lower jaw resection	–	Fixation of larynx, dysfunction of pharyngoesophageal phase	+	–	Delayed relaxation	0.5

*Therapy with botulinum toxin failed.

and dysfunction during the act of swallowing. For prognostic and diagnostic reasons, electromyography and magnetic stimulation of the paralyzed lower cranial nerves were performed.[13,14]

There were a total of 7 patients, 6 men and 1 woman, between 29 and 71 years of age. Two patients had a stroke with multiple dysfunctions and palsies, leading to complex dyscoordination and aspiration during swallowing. One patient had an acoustic neurinoma operation following unilateral palsy of the vagus and glossopharyngeal nerves. Three patients had previous tumor resections; 2 of them had tumors of the oropharynx with partial resection of the lower jaw, and 1 had supraglottic laryngeal carcinoma with partial laryngectomy. One patient had deglutition problems resulting from reflux disease with brachyesophagus. Three patients had to be nourished by a percutaneous gastric feeding tube. Six patients suffered from aspiration; 2 of them had a series of aspirations with resultant life-threatening pneumonias. All 6 patients with preoperative manometry and cineradiography had clear evidence of cricopharyngeal muscle (CM) dysfunction (Table 1).

Before operation all patients were informed about the procedure and possible side effects and gave written approval to the toxin injection. Follow-up was based on personal communication and chart review. Patients were instructed to return for their repeat injection when the effects of the previous injection began to diminish.

MATERIAL AND INJECTION TECHNIQUE

Botulinum toxin A was obtained from Dysport (United Kingdom) as a freeze-dried lyophilized preparation. For clinical use, botulinum toxin activity is defined in units, 1 unit representing the estimated median lethal dose for mice. Shortly before use, the toxin was dissolved with 2.5 mL of 0.9% sterile saline solution (without preservative), equivalent to 500 units.

To ensure precise localization, injections were always carried out under brief general anesthesia. Short-term muscle relaxants were used only in the introductory phase of the anesthesia, in order not to abolish electrophysiologic control. The CM can be adjusted best by rigid esophagoscopy using the specially designed Groningen esophagoscope,[15] which is a modification of the Holinger-Benjamin esophagoscope, with divided ends to easily depict the muscle bulge. Thereafter, a hooked wire electrode[16] for electromyographic control was inserted into the muscle,

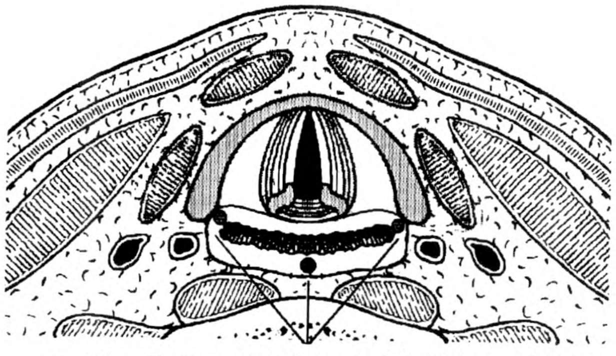

Fig 1. Localization of botulinum toxin injections. Administration of toxin into three different areas: into dorsomedial part and on both sides into ventrolateral part of cricopharyngeal muscle.

and the muscle activity was recorded simultaneously to the injection by using a Tonnies TESY II apparatus. Hyperactivity was recorded in all of our cases, with no relaxation period. For injection of the just-reconstructed toxin the diluted solution was drawn into a tuberculin syringe and administered through a butterfly cannula to three different areas: into the dorsomedial part and on both sides into the ventrolateral part of the CM (Fig 1). According to the severity of symptoms and the intraluminal pressure of the UES, the administered dose of botulinum varied between 80 and 120 units, equal to 0.4 to 0.8 mL of botulinum toxin solution (Table 1). The mean dose was 100 units per patient.

Patients were usually hospitalized for 2 days: the day of operation and the following day for observation. To achieve optimal efficiency, the amount of the injected dose of toxin was adjusted at each visit according to the patient's previous response.

TABLE 2. DISABILITY RATING SCALE

Score	*Level of Dysphagia*
0	Normal function, patient without complaints
1	No functional impairment, but subjective dysphagia when swallowing solid and/or liquid foods
2	Mild functional impairment for solid and/or liquid foods
3	Marked disability with moderate aspiration
4	Severe functional impairment with complete inability to swallow, considerable aspiration, and pneumonias

RESULTS

To better evaluate the surgical outcome of this study, we devised a disability rating scale for swallowing impairment. The severity of dystonia before and after injection was rated on a scale of 0 to 4 (Table 2). Rating resulted from subjective and objective parameters (postoperative manometric and cineradiographic control). Three patients clearly benefited from the procedure and were free of complaints thereafter. Aspiration problems stopped completely and there was no longer discomfort when swallowing solid or liquid food. These findings were supported by almost normalized postoperative deglutition shown on cineradiographic examination (Fig 2). Two patients mainly noticed improvement for solid food but continued to aspirate from time to time when drinking.

The treatment procedure was ineffective in 2 patients. The patient with reflux disease underwent

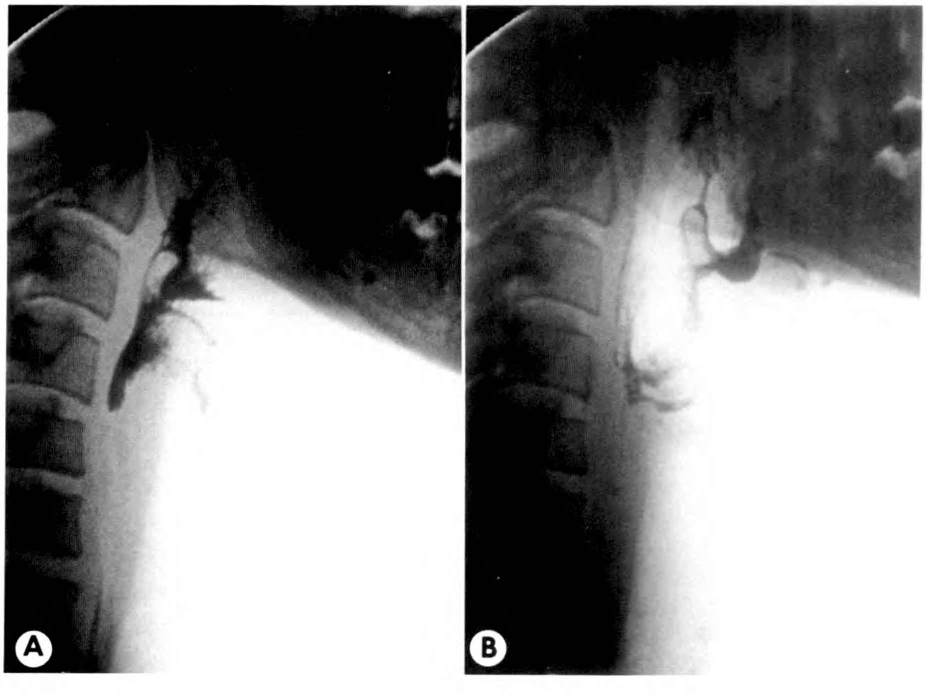

Fig 2. (Patient 1) Cineradiographs. Deglutition act of 29-year-old patient with dystonia of upper esophageal sphincter. A) Before treatment. Shows delayed relaxation of cricopharyngeal muscle and consequent aspiration. B) Normal act of swallowing, 4 weeks after injection of 0.4 mL of botulinum toxin.

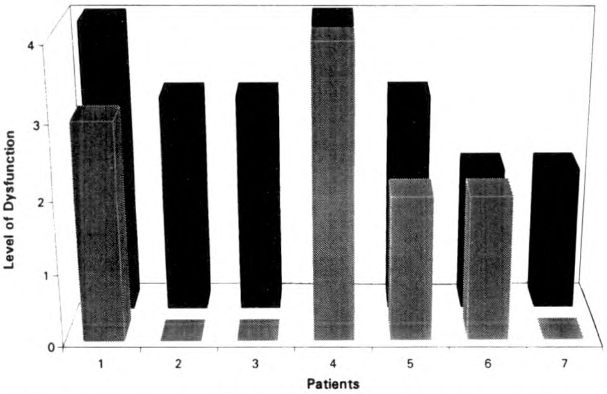

Fig 3. Summary of individual results of treatment with botulinum toxin. Solid bars show individual conditions before injection, and hatched bars show improvement after injection, defined by global rating on 0 to 4 disability scale (see Table 2).

endoscopic laser dissection[17] of the UES and was free of complaints thereafter. (In contrast to all postoperatively performed manometric examinations of the UES, which showed an atonic sphincter with a pressure gradient of not more than 8 to 10 mm Hg, this patient's UES continued to be hypertensive.) The other patient who failed to improve finally had to undergo total laryngectomy because he had severe persistent aspiration and pneumonia, and all other therapeutic steps failed. A summary of individual results is shown in Fig 3. Two patients who had required a feeding tube were able to have the tube removed after the procedure. The onset of effect was seen after approximately 8 days. The duration of maximum benefit lasted up to 5 months. No systemic effect has been seen or suspected in any of the patients, nor did any patient develop severe postoperative problems. Negative side effects were reported in only 1 patient: mild and transient heartburn not lasting longer than 14 days. Up to this point in the study 2 patients have returned, after time intervals of 4.5 and 5 months, and been injected again. One patient received the same dosage, and the other patient was injected with twice as much botulinum toxin to achieve a better response because of the severity of symptoms. None of our patients received other concomitant therapy.

DISCUSSION

Although the sample in this study is small, this form of therapy seems to be promising. The advantages of this method are low-risk intervention because of the brevity of anesthesia and the noninvasive character of the procedure (important for patients in poor general condition). This procedure leaves no scar; neither is it painful. Therefore, some of our patients choose the toxin as a definitive therapy, despite the temporary nature of its effects. Disadvantages are, of course, the temporary effect and the necessity of repeated injections at intervals of about 5 months. Sometimes more than one procedure may be needed to define the optimum dose and thus obtain maximal benefit. The blind transcutaneous approach, as suggested by other authors, does not allow a precise infiltration at various points of the CM, and also could cause an additional swallowing problem by affecting the constrictor muscles of the pharynx.

SUMMARY

Localized injection of botulinum toxin into the CM proved to be a relatively safe and effective alternative treatment for selective denervation of the UES. It works for patients with isolated hypertonus of the UES and for most patients with severe and complex disorders of the swallowing act combined with aspiration problems. Because of the temporary effect, we advocate this therapy as a method of first choice for transient disorders (for patients with nondegenerative palsies of the lower cranial nerves) and as a screening method (before cricopharyngeal dissection), to find out if cricopharyngeal myotomy[18] can be successful (especially in patients with very complex disorders). These first demonstrations of chemical denervation of the CM open up new possibilities for the application of botulinum toxin in clinical practice. A future intervention will be to apply the toxin transcutaneously, directly into the CM without anesthesia and under radiologic control, which could also enable an exact application of toxin.

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CURRENT TRENDS, CONTROVERSIES, AND PERSPECTIVES IN RHINOLOGY AND SINUS SURGERY

The international symposium Current Trends, Controversies, and Perspectives in Rhinology and Sinus Surgery will be held April 14-16, 1994, in New York City. For further information, contact NYU Medical Center Post-Graduate Medical School, 550 First Ave, New York, NY 10016; telephone (212) 263-5295; fax (212) 263-5293.

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The Fourth Research Workshop on the Biology, Prevention, and Treatment of Head and Neck Cancer, sponsored by the American Society for Head and Neck Surgery and the Society of Head and Neck Surgeons, will be held in Arlington, Virginia, on September 8-11, 1994. For further information, contact We Plan Meetings, Inc, Ruth C. Enquist, President, 1503 15th St NE, Rochester, MN 55906; telephone (507) 285-1523; fax (507) 281-8328.

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COMPOSITE RECONSTRUCTION OF THE ESOPHAGUS AND HYPOPHARYNX AFTER SEVERE CAUSTIC INJURY

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The ingestion of caustic materials may cause burns and subsequent strictures of the esophagus. Depending on the extent of the injury, the stricture may require dilation, excision, or bypass with an esophageal replacement. There is also a risk of esophageal dysfunction, perforation, and malignancy. Here is presented a case of a burn so severe that the hypopharynx was deformed and the esophagus destroyed. Composite techniques restored complete gastrointestinal continuity while maintaining excellent swallowing function.

KEY WORDS — benign stricture, caustic injury, esophageal replacement.

INTRODUCTION

The ingestion of caustic substances is a dangerous accident that affects 5,000 children per year in the United States.[1] The peak age for this activity is between 2 and 3 years, the time when meddlesome toddlers attain the ability to wander.[2] One of the more serious complications of this injury is an esophageal stricture, occurring approximately 12% of the time.[3] There is much controversy about the techniques available to prevent this complication once the burn has occurred. Steroids, stents, and prophylactic dilation all have been employed.[4,5] To date, no single method offers an effective prevention of this problem. The most critical factor that predicts stricture formation is the extent and severity of the burn. Once a stricture develops, similar disagreement exists as to how this should be corrected. Dilations (antegrade, retrograde, or balloon) are widely used.[6-8] Laser and diathermy are endoscopic techniques that are common. In those situations in which endoluminal techniques are not successful, open procedures such as resection, esophagoplasty, and esophageal substitu-

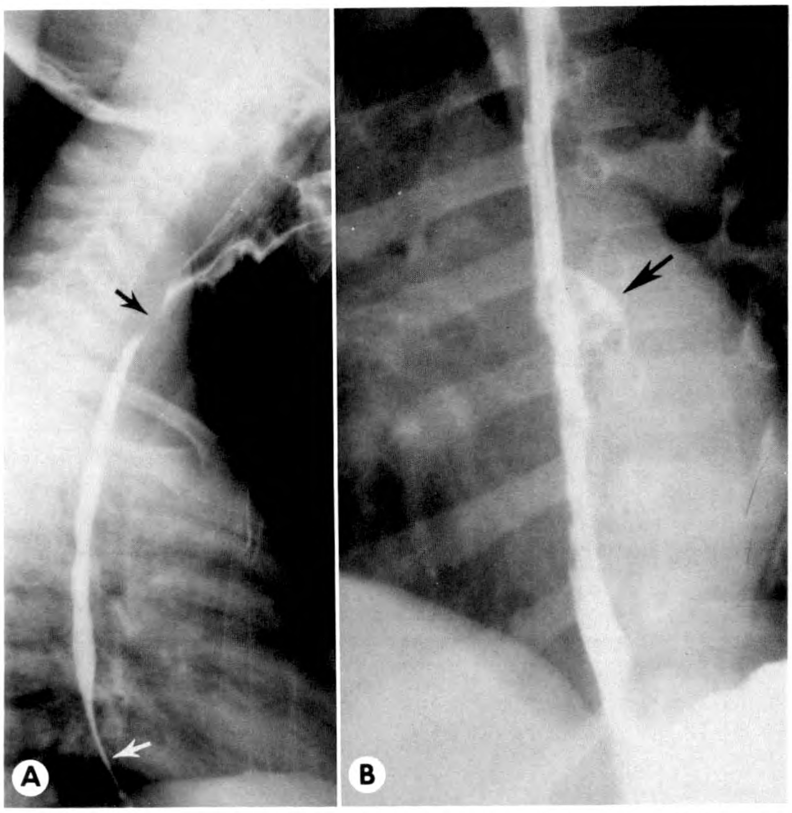

Fig 1. Esophagrams. A) Showing multiple narrow strictures, especially in cervical esophagus (arrows). B) Taken night after dilation and showing extravasation of contrast (arrow), confirming perforation of esophagus.

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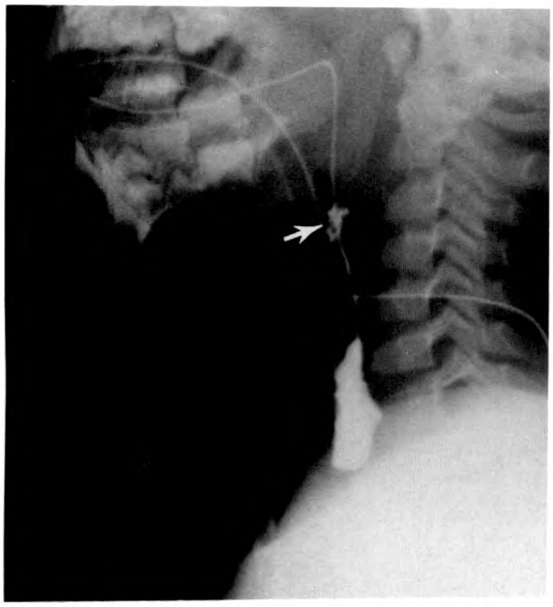

Fig 2. Esophagram of cervical esophagus and hypopharynx. Most severe stricture was at level of glottis (arrow). Reprinted with permission.[1]

tion are options.[9] In the present case the hypopharynx and esophagus were extensively damaged. No single operation would restore the ability to swallow and eat. By means of a combination of techniques, a functional conduit was achieved.

CASE REPORT

The subject was 2 years old when he accidentally ingested a solution of potassium hydroxide. His immediate condition was critical, with extreme airway compromise due to edema, emesis, and drooling of saliva and blood. He underwent emergency endotracheal intubation to control the airway. Endoscopy at that time revealed severe burns to the lips, tongue, palate, epiglottis, hypopharynx, and larynx. Because of the severity and the extent of the burns, esophagoscopy was not carried out past the cricopharyngeal muscle. The child was treated with antibiotics and corticosteroids for 14 days. During this time he was safely extubated and seemed to fully recover. When he clinically was able to swallow his saliva, an esophagram was obtained that showed good patency of the esophagus.

Several weeks later the child had trouble swallowing and began to spit and drool. A repeat barium swallow study demonstrated severe esophageal strictures (Fig 1A). To facilitate treatment and allow for feeding, a Stamm gastrostomy was placed. He underwent one antegrade esophageal dilation by means of string-guided dilators. That night a fever prompted an emergency repeat esophagram that revealed a rupture of the midthoracic esophagus (Fig 1B). He underwent emergent right thoracotomy in an effort to perform immediate repair of the esophagus. At the time of operation the area of rupture was found just inferior to the carina. However, it was also apparent that the esophagus had been severely injured and the muscular organ was replaced with a fragile membranous scar. It was felt that this could not be repaired and so the mediastinum was drained. To divert the saliva, a left cervical esophagostomy was fashioned. The child recovered from this and was supported with gastrostomy feedings.

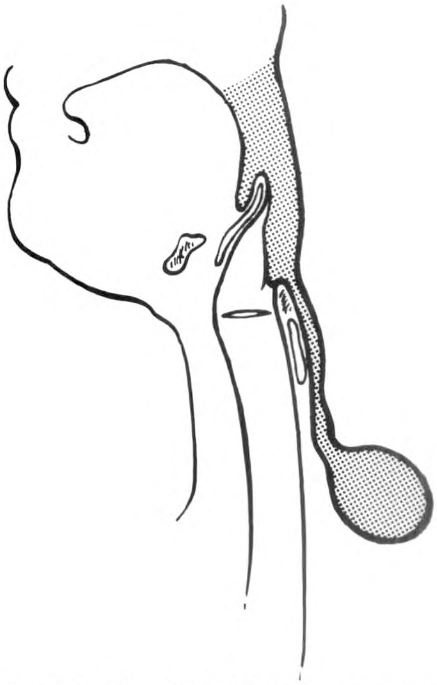

Fig 3. Rendition of condition of hypopharynx and cervical esophagus before reconstruction. This is compiled from radiography and endoscopy. Note that tightest stricture is high in hypopharynx.

Over the following 4 months the drainage from his cervical esophagostomy stopped, the skin healed over, and the child was not able to swallow. Endoscopy was performed, with an intraoperative contrast study demonstrating the stricture of the hypopharynx (Figs 2[1] and 3). The diameter of the esophagus and the hypopharynx at the level of the hypopharynx was less than 2 mm, admitting only a 3F catheter. The patient had to spit to eliminate the accumulated saliva. He had no airway or pulmonary symptoms despite the obstruction and inability to swallow. It was felt that this stricture could not be safely or successfully dilated because of the extent and location of the injuries. The child went for 2 years with no further treatment.

He then underwent reconstruction of the pharynx and cervical esophagus using a superiorly based platysma myocutaneous flap (Fig 4). An elliptical skin graft was designed that was perfused by the

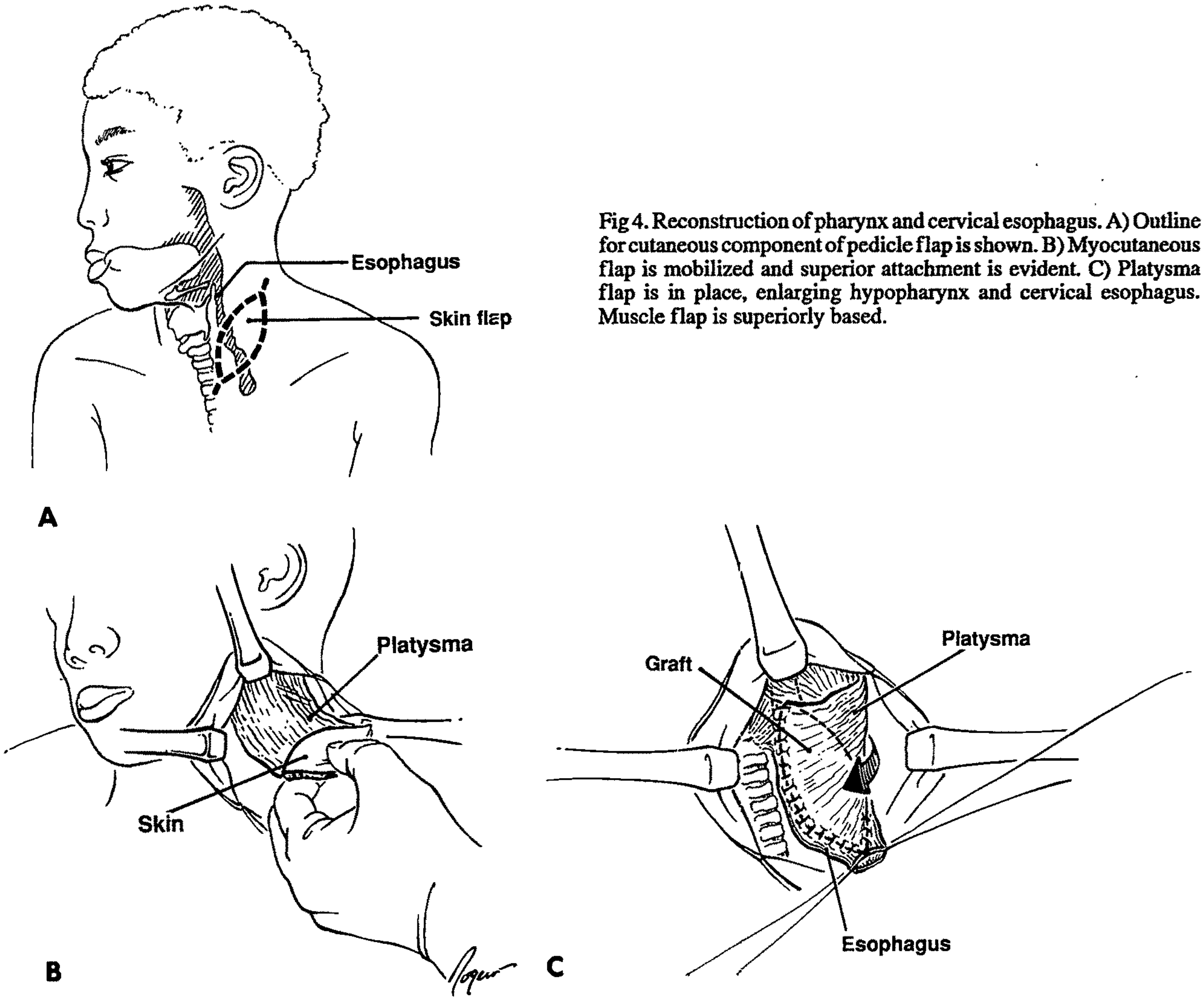

Fig 4. Reconstruction of pharynx and cervical esophagus. A) Outline for cutaneous component of pedicle flap is shown. B) Myocutaneous flap is mobilized and superior attachment is evident. C) Platysma flap is in place, enlarging hypopharynx and cervical esophagus. Muscle flap is superiorly based.

blood supply of the platysma muscle. The pedicle could then be rotated so the skin could be applied to the esophagoplasty. There was no compromise to or pressure on the pedicle. At the time of surgery several dense strictures were found. They were calcified, suggesting the presence of severe transmural injury and periesophageal inflammation. The skin flap was sutured in place with interrupted silk suture. This flap healed well and patency was assured with multiple dilations over the following year. When the lumen of the new hypopharynx and cervical esophagus had been stable for 1 year, the patient was begun on sham feedings.

At age 5 the patient underwent esophageal replacement using an ileocolic pedicle graft based on the ileocolic artery (Fig 5). The bowel segment was isolated and good blood supply confirmed. This pedicle graft was then positioned between the cervical esophagostomy and the stomach to ensure adequate length. This graft was ultimately placed substernally, with the vascular pedicle coursing through the lesser sac (Fig 6). In creating the cervical anastomosis, very little dissection was performed, so as not to disturb the platysma pedicle. Only the skin was completely dissected for closure. In placing the graft in the substernal location, the head of the clavicle and part of the sternum were excised to avoid obstruction. The patient recovered from this procedure without incident. Several weeks later a small cervical esophagocutaneous fistula was identified. This resolved spontaneously. He was ultimately left with an intact, functional conduit that allowed a complete swallow to the stomach. Radiographic studies showed good clearance during a swallow. After some training he was able to progress to regular food. His diet remains normal 3 years after his last operation. He has had no long-term complications. Ultimately his gastrostomy tube was removed.

DISCUSSION

Several characteristics of this case are unusual. First, potassium hydroxide is an uncommon household substance. Its characteristics as a base are simi-

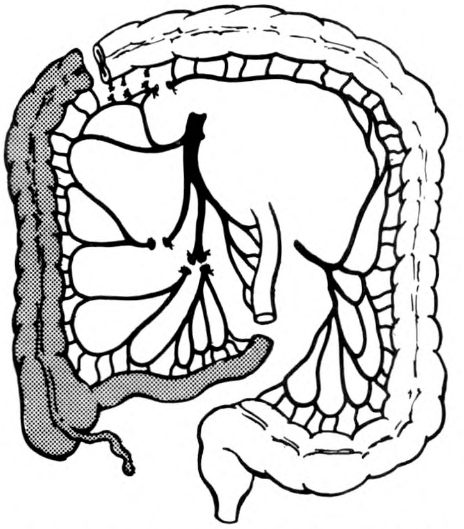

Fig 5. Ascending colon and terminal ileum are isolated for graft. Marginal artery is adequate here and pedicle is based on ileocolic artery. Note that by using ileocolic artery, extended ileal graft was possible.

lar to those of sodium hydroxide (see Table). In this case it functioned as a strong alkali, which is caustic to the esophagus. Poisoning from potassium overdose has not been observed, presumably because the amount of potassium in the solution is small. One hundred milliliters of 0.1 mol/L potassium hydroxide has 10 mEq of potassium, which is quite a small amount even for a rapid ingestion.

The burn and subsequent stricture formation were severe. In this situation there was a complete transmural destruction of the entire thoracic esophagus, leaving only the parietal pleura and scar. Thus, an esophageal substitution was required. The cervical esophagus was extensively scarred, resulting in multiple calcified strictures. The most severe was at the level of the inferior hypopharynx. It was felt that the hypopharynx should be reconstructed to preserve the swallowing mechanism. A platysma myocutaneous graft was used. As previously described, the blood supply of the platysma can support the skin graft.[10-12] There is the advantage of proximity and the minimal bulk produced by the platysma. Finally, as a pedicle flap, there was not the risk of delayed division of the blood supply to the graft. We modified the technique to use a superiorly based flap. There was no vascular compromise when a superiorly based flap was employed. The collaterals of the broad-based platysma, with the contribution of branches of the facial and occipital arteries, were adequate. Using this orientation, we were unencumbered when we interposed the ileocolic graft in the substernal location.[13,14] This technique requires excision of the left clavicular head and wide dissection of the neck and anterior mediastinum. Excising the clavicle and opening the inferior aspect of the neck would have interfered with an inferiorly based flap. Even after the pharynx was repaired, the cervical esophagus ended quite high (approximately C3-4). This required a substitute with considerable length. For this reason the ileocolic graft was chosen over the reverse gastric tube[15] or the descending colon. The ileocolic segment could be made longer by including more ileum based on the ileocolic artery. This made it possible to bring the graft for substitution much higher into the neck. This was important because the cervical esophagus was short, and since a platysma graft was in place, the cervical esophagus could not be mobilized. It was felt that the combination of this problem and the longer thorax of a child this age made a reverse gastric tube, gastric pull-up,[16] or descending colon a less desirable choice. Had the graft been inadequate, a microvascular free flap would have been an option.[17,18]

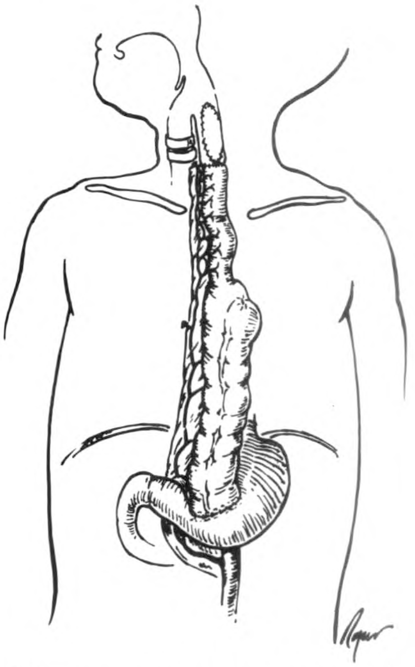

Fig 6. Entire reconstruction is pictured. Ileocolic graft is anastomosed to composite cervical esophagus anterior to stomach. Vascular pedicle is coursed through lesser sac. Conduit is in substernal position.

The treatment of the thoracic esophagus deserves comment. This organ was completely destroyed and

PROPERTIES OF SODIUM AND POTASSIUM BASES

	Molecular Weight	*Solubility (g/100 mL)*	*Median Lethal Dose (g/kg)*	*pH (0.1 mol/L)*
Potassium hydroxide	56.11	107	1.23	13.5
Sodium hydroxide	40.01	42	0.5	13

no patency at all could be demonstrated. Statistics suggest that this damaged esophagus is at a higher risk of developing carcinoma.[19-21] However, the extent of the burn made the esophagus difficult to identify and impossible to manipulate. It was felt that it could only be resected with extensive dissection, and that even then, complete removal of all precancerous cells could not be ensured. The risks and complications of resection outweighed the benefit of prophylactic esophagectomy. When the esophagus can be identified and resected with either the open or blunt technique,[22] then esophagectomy should be performed.

Injuries this extensive and severe create a complex set of problems. The area to be bypassed is of great length, and the injury potentially compromises the swallowing mechanism. Composite reconstruction offers a viable option. However, these techniques must be compatible both in function and anatomic orientation. This combination was performed successfully with few complications and a good long-term result.

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HEMODYNAMIC CHANGES IN THE HEAD AND NECK AFTER LIGATION OF THE UNILATERAL CAROTID ARTERIES: A STUDY USING COLOR DOPPLER IMAGING

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For evaluation of the hemodynamics of the collateral circulation to the ligated external carotid artery (ECA) region, we measured the blood flow direction and volume in branches of the ECA in patients with unilateral carotid artery ligation before and during digital suppression of the common carotid artery (CCA) on the ligated and nonligated sides with color Doppler imaging and angiography. The main collateral pathway to the ligated ECA region was the ipsilateral occipital artery through Richter's anastomosis from the vertebral artery in the case of unilateral ECA ligation, and was the contralateral carotid artery in the case of unilateral CCA, ECA, and internal carotid artery resection. The superior and inferior labial arteries were important as the collateral pathway from the contralateral ECA.

KEY WORDS — carotid artery ligation, collateral circulation, color Doppler imaging, head and neck surgery.

INTRODUCTION

Ligation of the external carotid artery (ECA) has been practiced in preparation for surgery of maxillary cancer and nasopharyngeal angiofibroma and is used in controlling severe nasal bleeding. Ligation of the common carotid artery (CCA) has been performed in patients with metastatic neck tumor involving the CCA or the internal carotid artery (ICA) and in those who have rupture of the CCA or the ICA. Recently, extensive radical neck dissection with resection of the CCA or the ICA followed by reconstruction of the carotid artery has been tried for patients with advanced head and neck cancer.[1] It is important for diagnosis and treatment to study the hemodynamic changes in the neck after ligation of the CCA, ICA, or ECA. Angiography, however, cannot quantify the hemodynamic changes, ie, blood flow and velocity.

Color Doppler imaging can demonstrate in real time, noninvasively and two-dimensionally, the soft tissue and blood flow. It can also quantitatively measure blood flow velocity. In this method, blood flow is displayed by colors: red indicates antegrade flow toward the ultrasonic probe, and blue indicates retrograde flow away from the probe. This method was useful for diagnosis in the head and neck region.[2,3]

We observed hemodynamic changes in the ECA region, including collateral pathways to the ECA, after unilateral ligation of the CCA, ICA, or ECA.

SUBJECTS AND METHODS

The subjects were 8 patients with unilateral ligation of the ECA and 3 patients with unilateral combined resection of the CCA, ICA, and ECA, and the study was performed more than 2 weeks after head and neck cancer surgery. The flow direction, velocity, and volume in branches of the ECA — ie, maxillary artery (MA), lingual artery (LA), and inferior and superior labial arteries (ILA, SLA) — before and during a digital compression test of the ligated or nonligated CCA were investigated by the color Doppler method. Collateral pathways to the ECA were confirmed by angiography. An SSA-270A color Doppler system with a 3.75-MHz convex probe and a 3.75- or 2.5-MHz linear array probe (Toshiba Co, Ltd) and an EUB 516 or EUB 565 color Doppler system with a 7.5-MHz linear probe and a 6.5-MHz convex probe (Hitachi Co, Ltd) were used for this investigation.

A sample volume of pulsed Doppler wave was placed in the blood vessel of interest, the angle marker was set to the direction of the blood flow to correct an angle factor, and then the Doppler shift was measured. The blood flow velocity waveform was calculated from the Doppler shift by a microcomputer within the equipment and visualized on a display. The maximum flow velocity of the waveform was traced by a digitizer, and the mean blood flow velocity was calculated. The cross-sectional area of the vessel was measured on a color image. Then the

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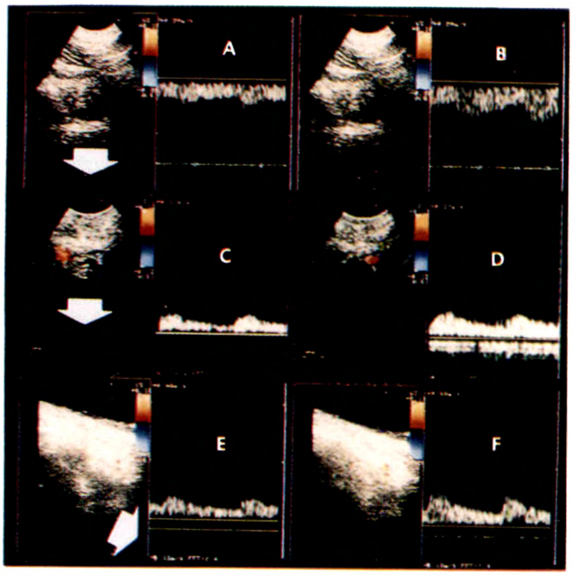


Fig 1. Color Doppler findings of branches of external carotid artery before and during compression of common carotid artery on ligated side in case of unilateral external carotid artery ligation. A) Maxillary artery before compression. Arrow — medial direction. B) Maxillary artery during compression. C) Lingual artery before compression. Arrow — caudal direction. D) Lingual artery during compression. E) Inferior labial artery before compression. Arrow — lateral direction. F) Inferior labial artery during compression. Waveforms are blood flow velocities. Blood flow velocity and volume increased, and direction of blood flow in arteries was antegrade with compression.

blood flow volume was calculated from the mean blood flow velocity and the cross-sectional area of the vessel.

RESULTS

Ligation of Unilateral ECA. The direction of blood flow in the MA, LA, and ILA on the ECA-ligated side before compression of the CCA on the ECA-ligated side was antegrade in all cases. On compression of the CCA on the ECA-ligated side, the blood flow velocities and volume increased (ranging from 19% to 41% of the value before the compression) and the direction of the blood flow in the arteries was antegrade (Fig 1). Regurgitation was detected from the occipital artery (OCA) on the ECA-ligated side to the ligated ECA in all cases (Fig 2).

The vertebral artery (VA), OCA, ECA, and MA were visualized in this order by VA angiography on the ligated side (Fig 3A). The facial artery (FA) and LA on the nonligated side were visualized by CCA angiography on the ligated side (Fig 3B).

Resection of Unilateral CCA, ECA, and ICA. The direction of blood flow in the MA, LA, and SLA on the resected side was retrograde. The blood flow volume of these was decreased by 100% in all cases during compression of the nonresected CCA. The blood flow volume of the SLA on the resected side was decreased by compression of the ILA or SLA on the nonresected side (Fig 4). The direction of the blood flow in the ophthalmic artery on the resected side was antegrade in two of three patients and retrograde in one.

Vertebral artery angiography on the resected side failed to visualize the branches of the ECA on the resected side, but CCA angiography on the nonresected side visualized the MA on the resected side (Fig 5).

DISCUSSION

Ligation of the ECA has been a conventional procedure for the head and neck surgeon. However, it does not always result in complete hemostasis, and some patients who have ligation of the CCA remain asymptomatic without reconstruction of the CCA. These phenomena are explained by the fact that the ECA and ICA regions are supplied by collateral blood flow in the neck after being ligated or resected.[4-6]

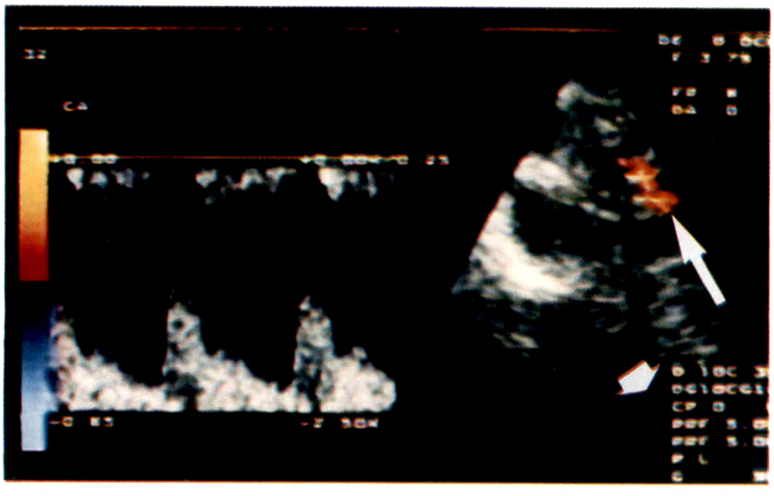


Fig 2. Color Doppler finding of regurgitation in occipital artery to ligated external carotid artery on external carotid artery–ligated side. Blood flow of occipital artery is shown by red (thin arrow). Thick arrow — cephalad direction.

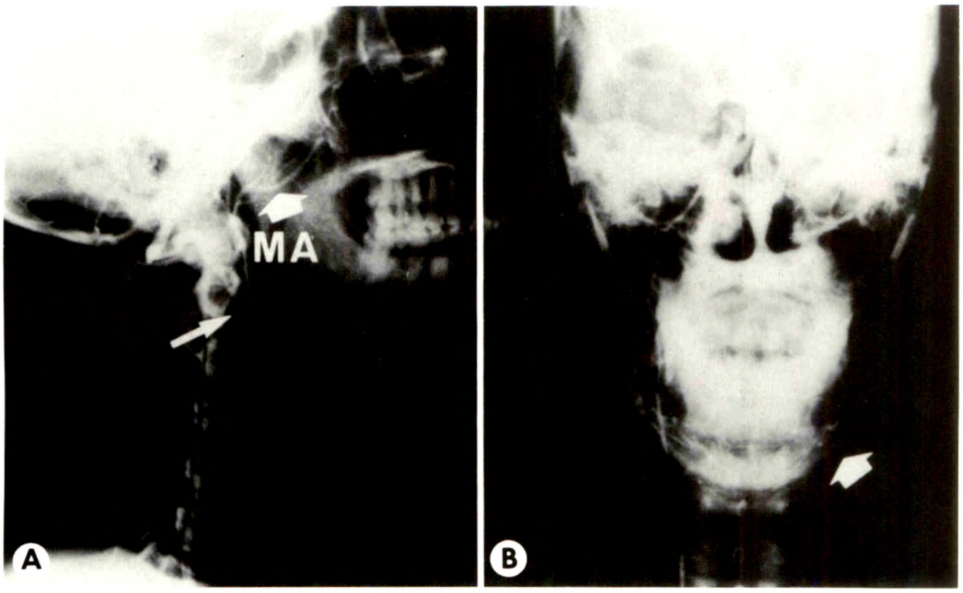


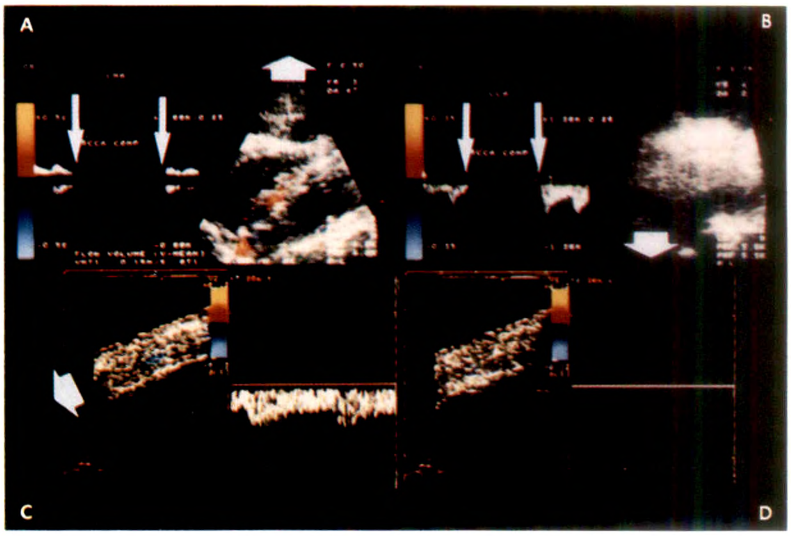
Fig 3. Angiogram in case of unilateral external carotid artery ligation. A) Vertebral artery, occipital artery (thin arrow), external carotid artery, and maxillary artery (MA; thick arrow) on ligated side were visualized in this order by vertebral artery angiography on ligated side. B) Facial artery and lingual artery on nonligated (left) side (thick arrow) were visualized by common carotid artery angiography on ligated (right) side.

Five collateral pathways to the ECA are known (Fig 6).[4-9] Hamanoue[5] studied the collaterals after ligation of the ECA using an acrylate-resin cast in dogs and described two important routes, ie, one from the ICA on the ligated side to the external ophthalmic artery (corresponding to the ophthalmic artery in humans) and further to the ECA on the ligated side, and one from the ECA on the nonligated side to the ECA on the ligated side. He also described a vertebral-occipital anastomosis on the ligated side. From these findings, he speculated that in humans, three collateral routes were important, one from the ophthalmic artery on the ligated side to the ligated ECA (route 2 on Fig 6), one from the nonligated ECA (route 1), and one from the subclavian artery via the deep cervical artery and the OCA to the ligated ECA (route 4).

Gandhi and Oppenheimer[4] studied the collateral pathways to the brain after ligation of the CCA and noted the importance of blood flow from the contralateral ICA via the circle of Willis (route 3) and from the bilateral VAs. Further, they pointed out the presence of anastomoses between branches of the bilateral ECA (route 1) and between the ophthalmic and angular arteries (route 2). They especially placed importance on anastomoses between the inferior and superior thyroid arteries and from the costocervical artery to the deep cervical artery and to the occipital artery (route 4).[4] Hamanoue[5] and Gandhi and Oppenheimer,[4] however, did not mention the importance of the anastomosis between the OCA and the VA.

Schulze and Sauerbrey,[10] in a report based on autopsies of 53 cases, reported the presence of an anastomosis between the OCA and the VA in all cases. Richter[8] also demonstrated this anastomosis in 25 of 30 cases by VA angiography. It has been called

Fig 4. Color Doppler findings of branches of external carotid artery on ligated side before and during compression of nonresected common carotid artery in case of unilateral resection of external carotid artery, common carotid artery, and internal carotid artery. Directions of blood flow in A) maxillary artery, B) lingual artery, and C) superior labial artery on resected side were retrograde. Their blood flow volume was decreased by 100% in all cases during compression test on nonresected common carotid artery (between two thin arrows). Blood flow volume of superior labial artery on resected side was decreased by compression of inferior labial artery and superior labial artery on nonresected side (D). Thick arrow — lateral direction in A, caudal in B, and lateral in C, respectively.



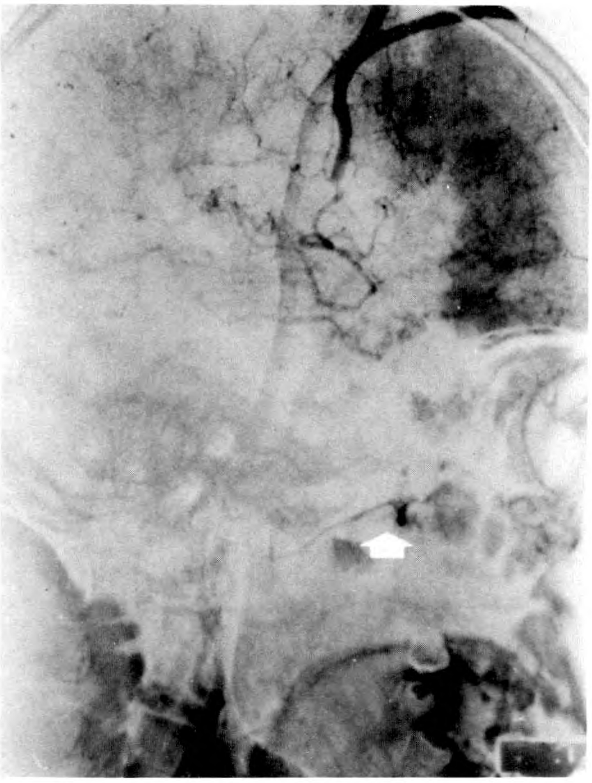


Fig 5. Common carotid artery angiogram on nonresected side in case of unilateral resection of external carotid artery, common carotid artery, and internal carotid artery. Maxillary artery on resected side was visualized (arrow).

Richter's anastomosis, and functions as the collateral pathway in occlusive disease of either the carotid artery or the VA. Therefore, it has been regarded as being as significant as the circle of Willis[7,9,11] (Fig 6).

Our results indicated that this anastomosis functions not only as a collateral pathway to the brain, but also as a collateral pathway to the neck and face region.

Our results indicate, in the patients with ligation of the unilateral ECA, that the main collateral pathways to the ECA were from the VA via Richter's anastomosis, and to the OCA as shown in Fig 6 (route 5).

According to our results, in the patients with resection of the unilateral CCA, ICA, and ECA, the blood supply to the resected ECA region depended on an intact ECA and ICA. The FA region on the ligated side, especially, depended on an intact FA through the SLA and ILA. If the blood flow in the ophthalmic artery on the resected side is antegrade, route 3 in Fig 6 will be regarded as the main collateral pathway to the ECA. If it is retrograde, route 1 will be.

Considering the collateral blood flow, ligation of the unilateral ECA is not necessarily an effective procedure to control severe nasal bleeding or bleeding in nasopharyngeal hemangiofibroma or maxillary cancer surgery.

The nasal cavity is supplied by two vascular systems, the ECA and the ICA. Therefore, ligation of both the MA (derived from the ECA) and the anterior or posterior ethmoid artery (from the ICA) is theoretically an effective procedure to control bleeding. However, hemostasis is still not complete, and one must consider the collateral flow from the MA on the opposite side.

In order to reduce blood loss during resection of nasopharyngeal hemangiofibroma or maxillary cancer, the blood flow from the ECA must be blocked just above the superior thyroid artery, and the collateral flow from the VA must also be blocked at the OCA. The same procedures on the intact side are necessary when the lesion receives a plentiful supply

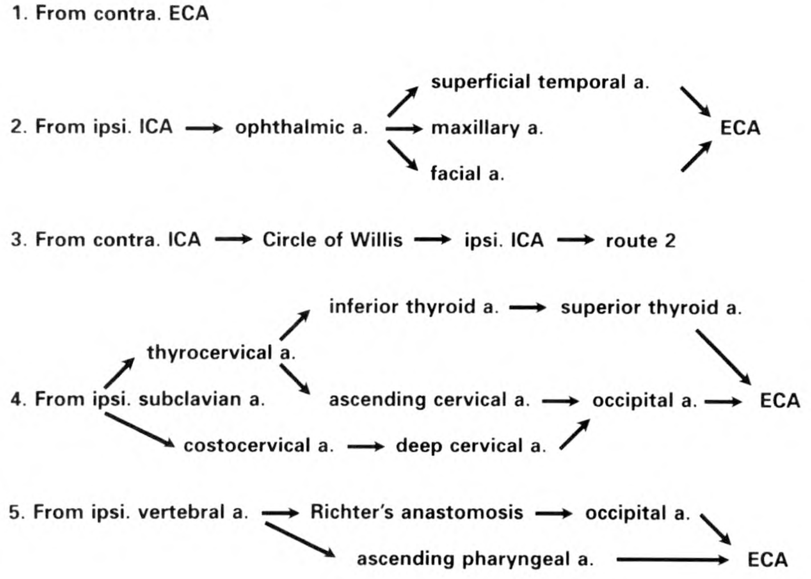

1. From contra. ECA
superficial temporal a.
2. From ipsi. ICA → ophthalmic a. → maxillary a.
facial a.
ECA
3. From contra. ICA → Circle of Willis → ipsi. ICA → route 2
inferior thyroid a. → superior thyroid a.
thyrocervical a.
4. From ipsi. subclavian a.
ascending cervical a. → occipital a. → ECA
costocervical a. → deep cervical a.
5. From ipsi. vertebral a. → Richter's anastomosis → occipital a.
ascending pharyngeal a. → ECA

Fig 6. Collateral pathways to external carotid artery (ECA). Contra. — contralateral, ipsi. — ipsilateral, a. — artery, ICA — internal carotid artery.

of blood from the branches of the ECA.

The hemodynamics in the neck after ligation or resection of the unilateral CCA, ICA, or ECA are different from those before surgery. In these situations, selective intraarterial infusion of an agent to the lesion requires a hemodynamic study of the neck.

Recently, resection of the primary lesion including the CCA and/or ICA after radiotherapy or chemotherapy has been attempted in order to cure advanced head and neck cancer. Pedicle or free myocutaneous flaps have been used in reconstruction of surgical defects. Flap viability is influenced not only by the blood flow of the feeding arteries or veins, but also by the blood flow of the recipient site. It is desirable, in making flaps, to consider the hemodynamic changes of the recipient sites.

Ligation of the CCA, ICA, or ECA can be performed relatively safely and effectively with regard for the collateral pathways in the head and neck. In patients who have ligation of the CCA, ICA, or ECA, it is also important to know the hemodynamics of the head and neck region, which provides useful information for intraarterial chemotherapy or reconstructive surgery.

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CONFERENCE ON AUDIOLOGICAL MANAGEMENT OF THE ELDERLY

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LYMPHATIC DRAINAGE SYSTEM AFTER LEFT RADICAL NECK DISSECTION

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The thoracic duct is the main lymphatic vessel that drains most of the body's lymph and all of the intestinal chyle into the venous system. The thoracic duct may be ligated during left radical neck dissection (RND) without any significant sequelae. The lymphatic system must, therefore, have collateral channels. The present study shows an increased incidence of pleural effusion after left RND when compared to right RND. We suggest that this represents the inability of the collateral system to immediately accommodate the increased volume of chyle.

KEY WORDS — chylothorax, neck dissection, thoracic duct.

INTRODUCTION

In order to avoid unnoticed injury to the thoracic duct during left radical neck dissection (RND), it is recommended that one avoid sharp dissection in the lower neck and ligate all soft tissues before dividing them. If the thoracic duct is identified, the surgeon can either try to preserve it, with a risk of an unnoticed injury, or relatively safely ligate it.

Where does the lymphatic system drain after ligation of the thoracic duct? A few years ago we described a case of chylothorax without external chylous fistula after left RND.[1] On the basis of that case and on extensive anatomic review, we concluded that collateral pleural, mediastinal, and bronchopulmonary lymphatic channels will provide drainage after thoracic duct ligation. Chylothorax may result from the inability of this collateral system to fully function immediately after surgery. To examine this hypothesis, we conducted a prospective study of 77 patients who had RND.

PATIENTS AND METHODS

Seventy-seven patients who underwent RND were included in the study. Sixty-nine patients had a known primary head and neck squamous cell carcinoma. Eight patients had cervical squamous cell carcinoma with an unknown primary. Excluded from the study were all the patients who had "prophylactic" or "elective" neck dissection, modified or selective neck dissection, or bilateral neck dissection, and patients who had extensive disease that required mediastinal or thoracic dissection. Patients who had external chylous fistulae were excluded from the study. Also excluded from the study were patients who had any radiographic evidence of preoperative pleural effusion, even a minimal one. All patients had complete classic cervical lymphadenectomy (levels 1 to 5) and removal of the internal jugular vein, the sternocleidomastoid muscle, and the 11th cranial nerve. The lower limit of the dissection was the clavicle. No matter on which side the neck dissection was performed, no sharp dissection was done in the lower neck; all soft tissues were double-clamped, divided, and ligated. No specific effort was made to identify the thoracic duct during surgery. However, if it was unintentionally exposed, as happened in 45.9% of left neck dissections, it was ligated. If a chyle leak was noticed during surgery, all efforts were made to identify the source of the leak and ligate and/or oversew it. Patients who had intraoperative evidence of chyle leak were given no food or water by mouth for a few days after surgery. If no external chyle leak was noticed, oral or tube feeding was started with a special formula containing medium-chain triglycerides and no or a minimal amount of long-chain triglycerides. Patients who had no intraoperative leak were fed on the first postoperative day.

The study group included 37 patients who had left RND. The control group included 40 patients who had right RND. All patients had anterior-posterior (and lateral if possible) chest radiographs on the third postoperative day. Every effort was made to bring the patient to the radiology suite for erect chest films. If this was not possible, chest radiography was performed on the ward with the patient in a sitting position.

RESULTS

In the study group, out of 37 patients who had left RND, 34 patients (91.9%) had postoperative radiographic evidence of pleural effusion. The effusion appeared usually as a minimal blunting of the

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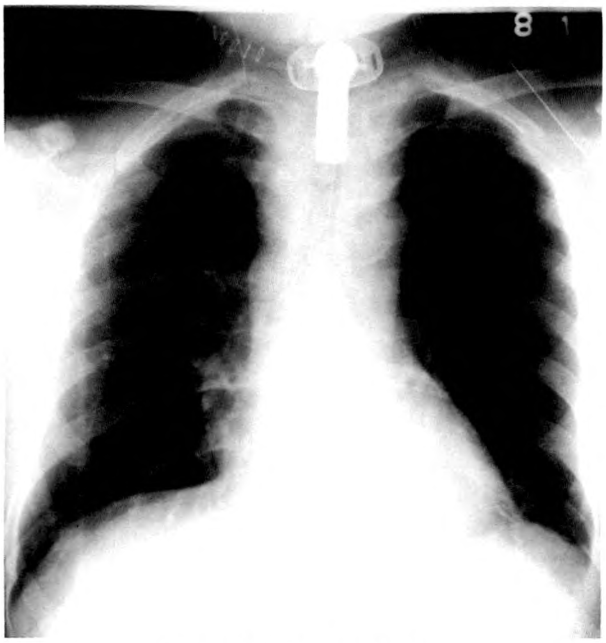

Minimal left pleural effusion.

costophrenic angle (see Figure). Only 3 patients had moderate-size effusion. The effusion appeared in the left pleural space in 31 of the 34 patients. In the remaining 3 patients the effusion was found bilaterally. None of the patients had an effusion that was significant enough to justify thoracentesis.

In the control group, out of 40 patients who had right RND, 1 patient (2.5%) developed postoperative *right* pleural effusion. Of interest, during the right RND that was performed on this patient who had squamous cell carcinoma of the base of the tongue, a large lymphatic vessel was found at the lower neck. This vessel, although not injured, was safely ligated. The difference in incidence of pleural effusion between the two groups was statistically significant ($\chi^2 = 61.85$, $df = 1$, $p < .0001$).

It took between 4 and 10 days for a complete resolution of the pleural effusion. All patients had complete disappearance of the effusion. There was no morbidity related to the pleural effusion in any of the patients.

ANATOMY

The thoracic duct arises from the cisterna chyli at the level of the second lumbar vertebra.[1-7] The duct ascends on the anterior surface of the vertebral bodies, between the aorta and the azygos vein. At the level of the fourth to the seventh thoracic vertebrae the duct crosses to the left, where it lies behind the aortic arch and the left subclavian artery. Having entered the base of the neck, the duct curves centrally to cross the anterior scalene muscle and the phrenic nerve. The termination of the thoracic duct in the neck is highly variable. It may terminate in the left internal jugular vein (the most common variant and the most common place for accidental injury during lymph node dissection resulting in postoperative external chylous fistula), left subclavian vein, left external jugular vein, left innominate vein, or right jugular vein. Parsons and Saargent[8] have made the observation that in 45% of the specimens they examined, the duct had two terminations. The thoracic duct receives all the lymph of the body except that from the right hemithorax, right upper limb, and right side of the head and neck.

PHYSIOLOGY

Chyle volume and flow rate may vary under different physiologic conditions. The normal daily volume is 1,500 to 2,400 mL, but in certain pathologic conditions, such as cirrhosis, the amount of chyle passing through the duct daily may reach 8,000 mL. A fatty meal provides the maximum increase in the flow of chyle.[3-5,7]

Chyle is a sterile, milky-appearing, odorless fluid having a variable leukocyte count, with lymphocytes predominating. The total fat content is high, ranging from 0.4% to 6%, depending on the diet. The main lipid component is triglycerides. The fat globules characteristically stain with Sudan red. Fat-soluble vitamins are also present in the chyle. The electrolyte composition is similar to that of the plasma, and the protein content, predominantly albumin, is generally 40% to 80% of the plasma values. The glucose levels are roughly the same as the serum values.[3-5,7]

According to Hesseling and Hoffman,[7] who described a rich collateral system, a simple obstruction of the thoracic duct hardly ever causes significant, symptomatic chylothorax. This collateral network allows chyle to find its way to the posterior intercostal lymphatic channels, the right bronchomediastinal trunk, and other lymphaticovenous shunts between the thoracic duct and the azygos, intercostal, and lumbar veins.

DISCUSSION

The results of our prospective study showed that there is an increased risk for small pleural effusion after left RND. Although thoracentesis was not performed on these patients and therefore we cannot state undoubtedly that the effusion was indeed a chyle effusion, we see no other possible explanation for the high incidence of effusion after left neck dissection and the very minimal incidence of effusion after right neck dissection, except for the possibility that this is related to the thoracic duct and the chylous system. The fact that the only patient who had right RND and had postoperative pleural effusion had also a large right cervical lymphatic vessel identified during surgery (most probably the right lymphatic duct) only supports the theory that these effusions are chylous in nature.

What is, then, the pathophysiologic mechanism leading to chylothorax without any sign of intraoperative or postoperative duct injury and/or external chyle leakage?

The pathophysiologic basis of chylothorax after left RND may be explained by the following.

1. The thoracic duct is ligated during the operation in the base of the left side of the neck.

2. Starting immediately, there is an increasing backward hydrostatic pressure in the thoracic duct.

3. The ability and capacity of the collateral lymphatic network to tolerate an increased volume and pressure of chyle vary from patient to patient.

4. The intraluminal pressure in the thoracic duct increases to such a point that nontraumatic extravasation of chyle is created.

5. Because of the negative pressure that exists in the thoracic cage during inspiration, the extravasation point is lower in the mediastinum than in the neck. This may explain the absence of cervical chyloma and/or external chylorrhea.

6. As chyle accumulates under pressure, it finally extravasates through one or both mediastinal pleurae, producing chylothorax. No direct injury to the pleurae is needed to produce chylothorax, but only high hydrostatic pressure and maceration of the pleural tissue by the chyle. This fact explains the absence of pneumothorax.

This was the original explanation for chylothorax without external chylous leakage.[1] However, the fact that our study shows that most of these patients have left, but not bilateral, chylothorax requires explanation. As hydrostatic pressure in the collateral channels increases, chyle is extravasating directly from left pleural lymph vessels into the left pleural space. Thus, chylothorax is produced without chyle accumulation in the mediastinum. The rate of absorption and disappearance of the pleural effusion is dependent on the patency of collateral channels for chyle flow. Early development of patent collateral channels may explain the short course and the relatively small amount of pleural fluids in our patients.

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COMPARISON OF CYTOPHOTOMETRIC CHARACTERISTICS TO HISTOLOGY AND PROLIFERATION MARKERS IN ACOUSTIC NEUROMAS

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Specimens of histologically confirmed acoustic neuromas obtained during operation in 25 patients were examined. Quantitative DNA measurements were performed with an image analysis system. From the single cell measurements, the mean DNA content of all tumor cells, 2c deviation index (2c DI), DNA entropy, DNA grade of neoplasia, and percentage of tumor cells with a DNA content of more than 5c (5c exceeding rate) were derived, as well as the mean nuclear area of the tumor cells. Proliferating cell nuclear antigen (PCNA) was identified immunohistochemically. A PCNA score was developed in determining PCNA-positive cells in a total amount of 1,000 cells. Results of quantitative DNA measurements and PCNA scores were compared to clinical symptoms, histology, and time between first onset of symptoms and diagnosis of the tumor. Quantitative DNA measurements revealed the existence of hyperdiploid tumor cells in all neuromas. According to the frequency with which they occurred, tumors could be divided into two categories: 1) tumors with a high percentage of hyperdiploid cells ("hyperdiploid tumors") and 2) those with a low percentage ("diploid tumors"). Hyperdiploid tumors showed increased values for the 2c DI, mean DNA content, DNA grade of neoplasia, and DNA entropy as signs of increased proliferation. In addition, the PCNA scores were higher in these tumors, indicative of increased DNA synthesis. The mean nuclear area was higher in these tumors. No correlation was found between the results of the DNA analysis and the PCNA score, or the clinical data and the predominant histologic subtype. The results of this study could explain the known differences in growth rate of acoustic neuromas and might also have clinical relevance in identifying patients at high risk for developing tumor recurrences.

KEY WORDS — acoustic neuroma, DNA content, hyperdiploid cells, immunohistochemistry, proliferating cell nuclear antigen.

INTRODUCTION

Acoustic neuromas are the most frequent benign tumors of the lateral skull base, having their origin in Schwann cells. More and more small tumors can be detected by means of evoked potentials and magnetic resonance imaging (MRI). Nevertheless, there have been few reports on the growth rate and proliferation of these tumors.[1-5] On the basis of past experience in determination of prognosis of epithelial lesions by DNA measurements and immunohistochemistry, it should be possible to prove whether those examinations are suitable for estimating the proliferation rate and are useful as prognostic indicators in acoustic neuromas. The purpose of this present study is to 1) correlate the results of quantitative DNA measurements and immunohistochemistry with history, clinical symptoms, and histology and 2) find out whether these tests have any prognostic relevance in estimating the proliferation and growth rates of acoustic neuromas.

PATIENTS AND METHODS

Specimens of 25 patients (12 men and 13 women, average age 56.8 years) who underwent surgery for an acoustic neuroma were examined. Fourteen patients had right-sided tumors; 11 patients had left-sided tumors. The specimens were assessed by routine histology, quantitative DNA measurements, and immunohistochemical determination for the proliferation marker proliferating cell nuclear antigen (PCNA). The results were compared to clinical data such as tumor size, medical history, and clinical symptoms.

QUANTITATIVE DNA MEASUREMENTS

For quantitative DNA measurements a single cell suspension was obtained from the paraffin-embedded specimens.[6] Fifty-micron sections were cut from this embedded tissue, deparaffinated twice in xylene, and rehydrated in 100%, 95%, 80%, 70%, and 50% ethanol for 10 minutes each. After two washings in phosphate-buffered saline (PBS; 50 mmol/L, pH 7.4, 10 minutes each), the cells were resuspended in 0.05% Pronase (Sigma Chemicals, St Louis, Mo) and incubated for 30 minutes at 37°C. The suspension was then filtered through a mesh with a pore size of 70 μm. The suspension was then centrifuged (3,000

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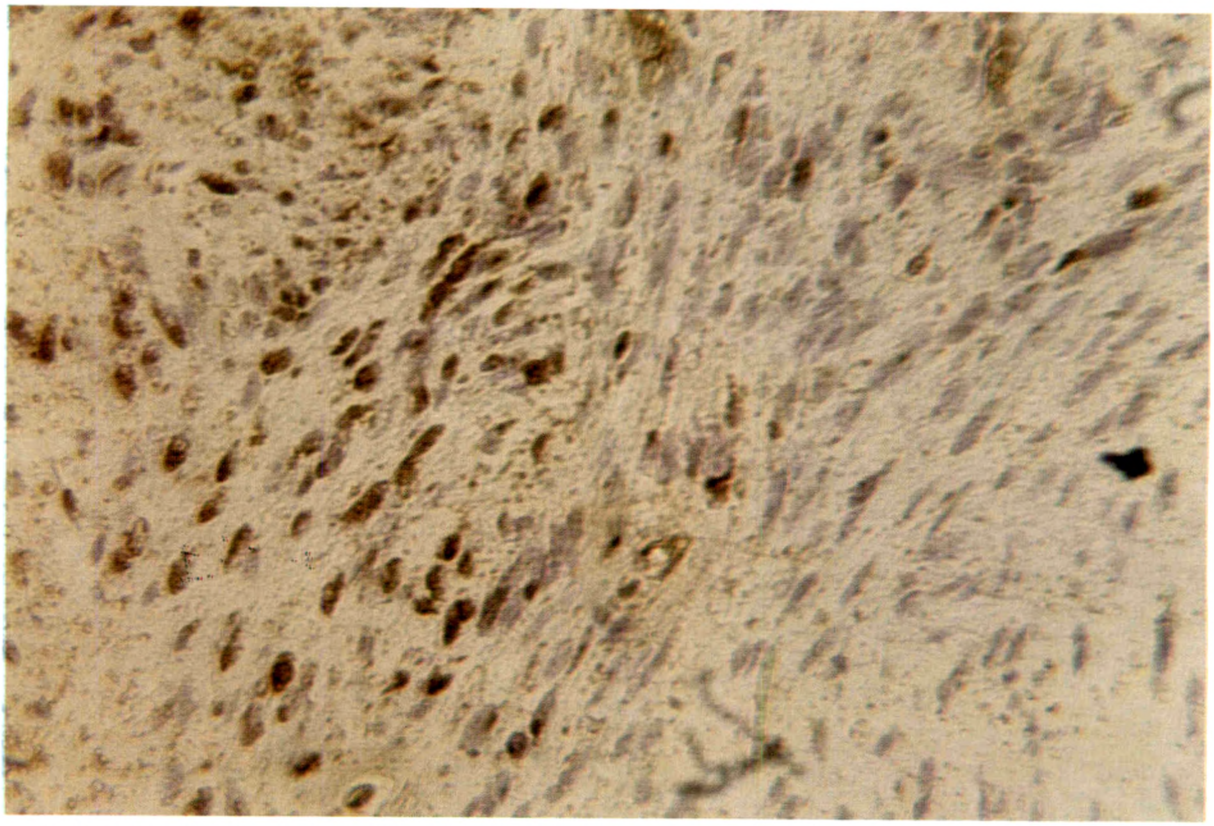

Fig 1. Immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in acoustic neuroma. Presence of PCNA leads to brownish color inside cell nuclei.

rpm for 10 minutes), resuspended in 3 mL Carbowax (2% polyethylene glycol in 50% ethanol; Merck Cie, Darmstadt, Germany), centrifuged again (1,500 rpm, 10 minutes), and then placed onto a glass slide. After air drying it was stained with fuchsin–sulfuric acid.[7] Quantitative DNA measurements were then performed with an automatic microscope with an attached video camera and a computerized image analysis system (Cytometer CM1, Hund Cie, Wetzlar, Germany).[8] At least 250 tumor cells were measured at random after morphological detection on a video screen. To determine the individual diploid 2c peak, at least 30 lymphocytes or granulocytes were measured in the same specimen. From the single cell measurements the following indices were determined.

1. The mean DNA content of the tumor cells.
2. The 2c deviation index (2c DI), defined as the mean square deviation from the normal diploid value of 2c.[9]
3. The number of cells with a DNA content of more than 5c (5c exceeding rate).
4. The DNA entropy.
5. The DNA malignancy grade.
6. The DNA content of the predominant stem cell line.
7. The number of hyperdiploid tumor cells.
8. The mean nuclear area of the tumor cells.

IMMUNOHISTOCHEMICAL DETECTION OF PCNA

Proliferating cell nuclear antigen is a nuclear protein with a molecular weight of 36 kD. It is identical with DNA-polymerase-δ-auxiliary protein and therefore essential for DNA synthesis.[10,11] This antigen is expressed in every phase of the cell cycle, with a clear peak in the S-phase. Therefore, it is suitable for determination of proliferation cells.[12] For immunohistochemical detection of this antigen a monoclonal antibody was used (Ab PC10 clone lane, Oncogene Science, Uniondale, NY). Four-micron sections of the paraffin-embedded specimens were taken. The sections were mounted on poly-L-lysine–coated glass slides, air-dried, dewaxed, taken through a graded alcohol series, and immersed for 10 minutes in 25% phosphate-buffered saline in methanol with 1% hydrogen peroxide to block endogenous peroxidase activity. Sections were then incubated with the antibody and stained by the avidin-biotin-peroxidase complex method.[13] The slides were assessed by counting the cells with positive PCNA immunoreactivity out of 1,000 cells. The percentage of PCNA-positive cells was called the PCNA score (Fig 1). Statistical analysis was performed with the Wilcoxon signed rank test.

RESULTS OF QUANTITATIVE DNA MEASUREMENTS IN ACOUSTIC NEUROMAS

	Diploid Tumors (n = 10)	*Hyperdiploid Tumors (n = 15)*
Mean DNA content of tumor cells	2.27 ± 0.28	2.81 ± 0.21
2c Deviation index	0.81 ± 0.66	2.71 ± 0.80
5c Exceeding rate (%)	1.26 ± 2.24	3.33 ± 2.42
DNA entropy	3.73 ± 0.27	4.18 ± 0.32
DNA grade of neoplasia	0.27 ± 0.23	0.67 ± 0.17

Data are mean ± 1 SD.

RESULTS

All 25 patients complained of hearing loss on the tumor side, 19 had ipsilateral tinnitus, and 14 complained of dizziness. The time between first onset of symptoms and operation averaged 2.47 ± 2.19 years (range 0.25 to 6 years). In each case, MRI was performed, and all tumors filled the internal auditory canal. Twenty-one tumors extended into the cerebellopontine angle (maximum extension of 22 mm). No correlation was found between the tumor size and duration or the severity of symptoms before diagnosis. Ten tumors were removed by a middle cranial fossa approach, and the remaining 15 were removed by translabyrinthine access. Histologically, all tumors were described as schwannomas, and they were histopathologically confirmed as benign. Characteristic cell patterns (Antoni types A and B) were observed in each specimen.

Quantitative DNA measurements revealed the existence of hyperdiploid tumor cells with large nuclei and compact chromatin structure in each tumor, but there was a remarkable difference in the frequency with which they occurred. According to this result, the tumors could be divided into two categories: 1) tumors with a rate of hyperdiploid cells of 16.6% ± 5.0% (range 11.0% to 27.5%; "hyperdiploid tumors" n = 15) and 2) tumors with a rate of hyperdiploid cells of 6.88% ± 2.11% (range 3.4% to 10%; "diploid tumors" n = 10). The results of quantitative DNA measurements in both groups are detailed in the Table.

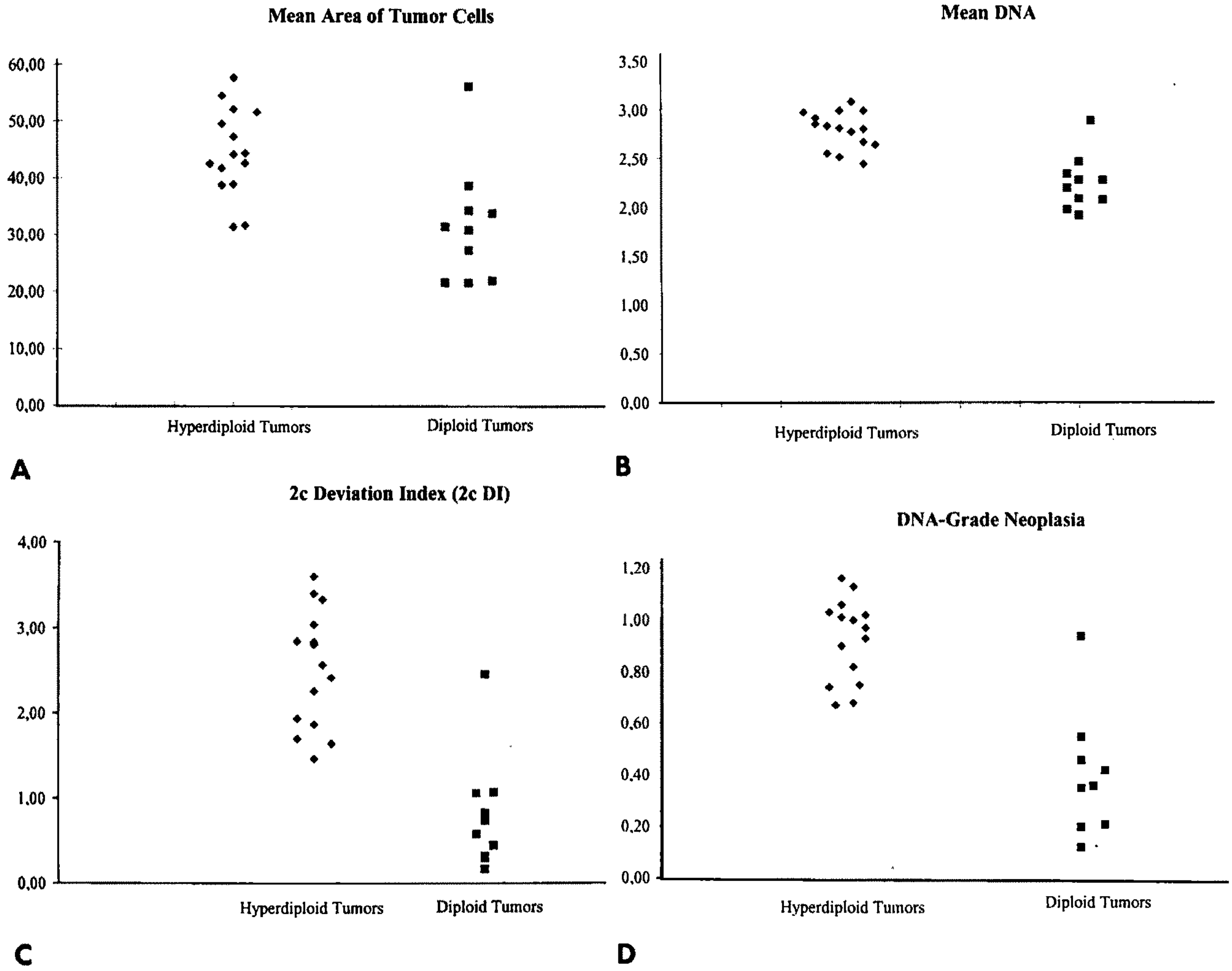

Fig 2. Scatter diagrams of A) mean area of tumor cells, B) mean DNA content of tumor cells, C) 2c deviation index, and D) DNA grade of neoplasia in diploid and hyperdiploid acoustic neuromas.

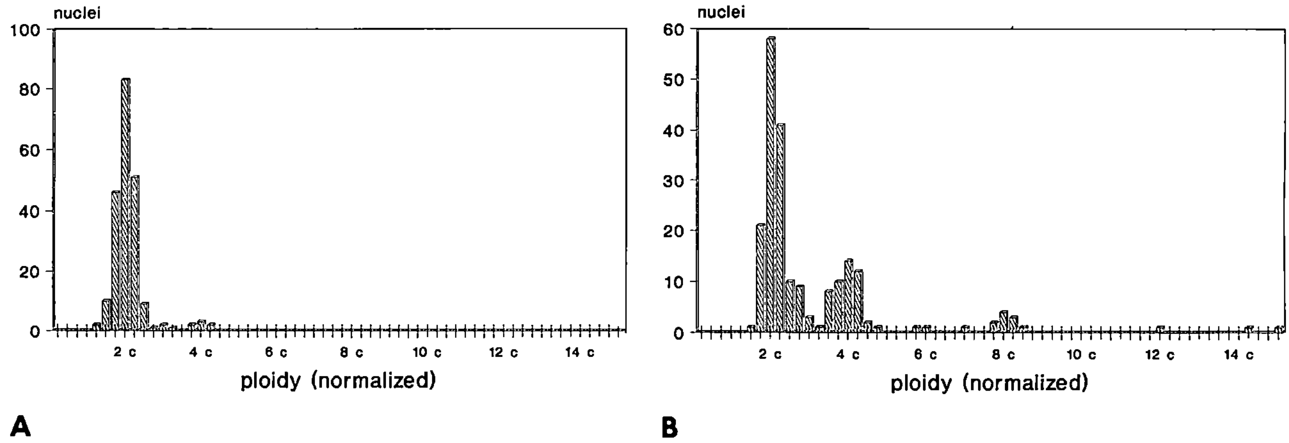

Fig 3. Examples of quantitative DNA measurements. A) Left-sided acoustic neuroma that consists primarily of diploid tumor cells. Proliferating cell nuclear antigen (PCNA) score is 3.2%. B) Right-sided, hyperdiploid acoustic neuroma. Note ploidy peaks at 2c, 4c, and 8c. In this case, PCNA score is 12.7%.

A comparison of the DNA data revealed statistically significant increased values for mean DNA content of the tumor cells ($p = .0042$), 2c DI ($p = .0018$), DNA grade of neoplasia ($p = .002$), and mean nuclear area ($p = .03$) in the hyperdiploid tumors as signs of increased proliferation activity in these tumors (Fig 2).

No correlation was found between these two tumor categories and predominant histologic type, time between onset of symptoms and diagnosis, or presence of clinical symptoms.

The results of PCNA scores showed an increased rate of PCNA-positive cells in the hyperdiploid tumors (8.7% ± 4.27%) when compared to the diploid tumors (6.2% ± 4.18%), indicative of increased DNA synthesis. Examples of quantitative DNA measurements in a hyperdiploid tumor and in a diploid tumor are illustrated in Fig 3.

DISCUSSION

Acoustic neuromas are present in approximately 1% of temporal bones, but there is a remarkable discrepancy in this number of tumors when compared with the number of these tumors that are diagnosed and treated.[5,14,15] There are only a few reports on the growth rate and proliferation of these tumors.[16,17] In all of these studies tumor growth rates were documented by a series of high-resolution computed tomography (CT) or MRI scans. Some authors suggested a correlation between tumor growth rate and the age of the patient, positing a slower progression in the elderly.[3,17-19] An initially small acoustic neuroma in a young adult was shown to progress rapidly during a 15-month period, as documented with serial high-resolution CT scans.[1] No correlation was found between the growth rate and the presence of clinical symptoms, histologic subtype of the tumors, or patient history in any of these studies. A review of the literature highlights the fact that two questions remain unresolved:

1. Why do some tumors become clinically apparent while others do not?
2. Why do some tumors have a more rapid progression while others remain dormant?

Experimental studies with implantation of surgically removed acoustic neuromas into the subrenal capsule of nude mice showed a close relationship between growth rate and neovascularization.[4] Consequently, neovascularization is one factor that tumor growth depends on. However, this cannot explain the reason why some tumors undergo periods of rapid growth while others do not. The reason for this could possibly be found on the molecular level of the tumor cells. In the present study 225 acoustic neuromas were examined by quantitative DNA analysis, which has proved prognostically significant in many human tumors, and immunohistochemical identification of PCNA, which is essential for DNA synthesis.[8,20] Thus, high levels of PCNA-positive cells in a tissue may be correlated with high proliferative activity.[12]

With quantitative DNA measurements, tumors with a high percentage of hyperdiploid cells and with an increased nuclear area can be distinguished from tumors that consist of diploid cells alone. Furthermore, hyperdiploid tumors have a higher PCNA score and represent some nondiploid tumor cells. The presence of nondiploid tumor cells is not limited to histopathologically malignant cells in nonepithelial tissue, as is known to occur with benign pituitary tumors.[21] One might infer that these tumors have a higher proliferative activity and therefore a higher growth rate. Patients with hyperdiploid tumors therefore need more careful follow-up in an attempt to detect possible tumor recurrence, which may be more likely in those cases. On the other hand, these findings may explain the observed different growth rates, and the fact that some tumors become clinically apparent while others remain dormant. Other researchers have reported on acoustic neuromas assessed by flow cytometry.[22] They found S-phase values reflecting the slow growth of these tumors. The highest S-phase value, however (8.3% of tumor cells in S-phase), was found in a tumor recurrence. In the present study we found S-phase values ranging between 2.02% and 12.9% by immunohistochemistry. Furthermore, the technique of quantitative DNA measurement used in this study is more subtle in detecting single nondiploid tumor cells than is flow cytometry.[20]

We were able to demonstrate a lack of correlation between the results of quantitative DNA measurements, PCNA score history, and clinical symptoms, as well as predominant histologic classification. This means there is currently no preoperative or morphological tool that can identify rapidly progressive tumors from those that have a lower rate of proliferation. Our conclusions reveal that some acoustic neuromas have a higher percentage of hyperdiploid tumor cells. In addition to the increased PCNA score, one might suggest a higher proliferative rate and a more rapid progression in these tumors. Consequently, the observed different growth rates in acoustic neuromas may possibly be due to these findings.

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DOES TOPICAL ANTIBIOTIC PROPHYLAXIS REDUCE POST–TYMPANOSTOMY TUBE OTORRHEA?

A META-ANALYSIS

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Purulent otorrhea is the most common complication of tympanostomy tube (TT) insertion. It may occur in the postoperative period or at any time during the sojourn of the tube. The efficacy of topical antimicrobial prophylaxis against purulent postoperative otorrhea (PPO) has been examined in 5 prospective, randomized studies; all demonstrated a reduction in PPO from topical antimicrobial prophylaxis, but in only 1 study was the difference statistically significant. Because the 5 studies used 2 different experimental designs — by-patient, and by-ear — a single meta-analysis could not be done. However, the by-patient studies met the criteria for meta-analysis, which demonstrated a combined odds ratio of 0.12 (95% confidence interval 0.04 to 0.37, p = .0002). This represents an 85% reduction in otorrhea, which is judged to be clinically as well as statistically significant. We conclude from the available evidence that prophylactic use of topical antimicrobial agents following TT insertion consistently reduces the rate of PPO. However, the low incidence of PPO and the heterogeneity of the published studies prevent making a final judgment for or against the continued use of these agents. Therefore, given that these potentially ototoxic agents are frequently administered to prevent postoperative otorrhea, further study of this subject is warranted. In the meantime, we recommend judicious use of these agents following TT insertion in those cases at higher risk for PPO, namely those with mucoid or purulent effusion.

KEY WORDS — meta-analysis, purulent postoperative otorrhea, topical antimicrobial prophylaxis, tympanostomy tube.

INTRODUCTION

Purulent otorrhea is the most common complication of the insertion of tympanostomy tubes (TTs) for treatment of chronic otitis media with effusion in children, with a reported incidence in the postoperative period ranging from 10%[1] to over 30%.[2,3] Delayed-onset otorrhea is a common problem for patients with TTs that requires medical therapy and, not infrequently, removal of the tubes. Although purulent postoperative otorrhea (PPO) generally responds promptly to treatment and serious consequences are uncommon, considerable expense and effort are expended to prevent and treat it.

Prophylactic therapy is used routinely after TT insertion by many otolaryngologists to prevent PPO. The most common form of prophylaxis is medical and employs one of several types of topical antimicrobial agents, even though the manufacturers specifically exclude use in the middle ear because of the risk of ototoxicity.

There is now uncertainty about the value of medical prophylaxis against PPO. Five randomized clinical trials[4-8] have examined the value of prophylaxis of PPO. All 5 studies demonstrated a reduction in the incidence of PPO from the use of topical antimicrobial prophylaxis, yet in only 1 of the studies was the effect large enough to reach statistical significance.[4] Because of a favorable anecdotal experience,[1] many surgeons continue to use topical antimicrobial prophylaxis after TT insertion, even though the number of negative studies is greater than the number of positive studies. Indeed, all of the negative studies[5-8] demonstrated a lower incidence of PPO in the treated cases than in the controls, but the incidence of PPO was small enough that a clinically significant effect may not have been detectable, and none of the reports included an estimate of the statistical power of the negative results. Thus, physicians and patients are caught in a dilemma concerning whether to use topical antimicrobial agents to prevent PPO. On the one hand, if the studies failed to demonstrate effectiveness because of flaws in experimental design, patients needing prophylaxis might not receive it. On the other hand, if there is truly no effect, then antimicrobial drops should probably not be used in the

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META-ANALYSIS OF EFFECT OF TOPICAL ANTIMICROBIAL PROPHYLAXIS ON RATE OF POSTOPERATIVE OTORRHEA

			Otorrhea Rate				
Study No.	*Reference*	*N*	*Treatment Group*	*Control Group*	*Odds Ratio*	*95% Confidence Interval (Lower and Upper Bounds)*	*p*
1	4	102	0/56 (0%)	9/46 (20%)	0.035	0.002, 0.618	.0007
2	5	60	2/34 (6%)	5/26 (19%)	0.263	0.050, 1.376	.222
3	6	35	1/18 (6%)	3/17 (18%)	0.275	0.028, 2.705	.338
Subtotal		197	3/108 (3%)	17/89 (19%)	0.121	0.040, 0.365	.0002
4	7	120	5/60 (8%)	8/60 (13%)	0.591	0.182, 1.914	.378
5	8	400	23/200 (12%)	26/200 (13%)	0.870	0.478, 1.583	.647

middle ear because of the risk of ototoxicity.

In an attempt to resolve this dilemma, we performed a meta-analysis of the existing studies to estimate the overall effect of topical antimicrobial prophylaxis against PPO.

MATERIALS AND METHODS

Literature Search. The Medlars (Medical Literature Analysis and Retrieval System) data base was searched by using Medical Subject Headings of tympanostomy, complications, and otorrhea. All articles so identified were investigated. In addition, standard textbooks were consulted for other references not identified in the Medlars search. Inclusion criteria were 1) a prospective, randomized clinical trial, 2) treatment duration of 48 hours or more, 3) treatment limited to topical antimicrobial agents, 4) subjects who were children with otitis media with effusion, and 5) an outcome measured within 1 month of the surgery. Nonrandomized and retrospective studies were excluded. Cases with concomitant use of oral antimicrobial agents were omitted from analysis.

Statistical Analysis. The research question asks whether topical antimicrobial prophylaxis reduces the incidence of otorrhea in the early postoperative period. The research hypothesis is that the rate of otorrhea in the control group and that in the treatment group are not different. All forms of topical antimicrobial prophylaxis were assumed to be equivalent.

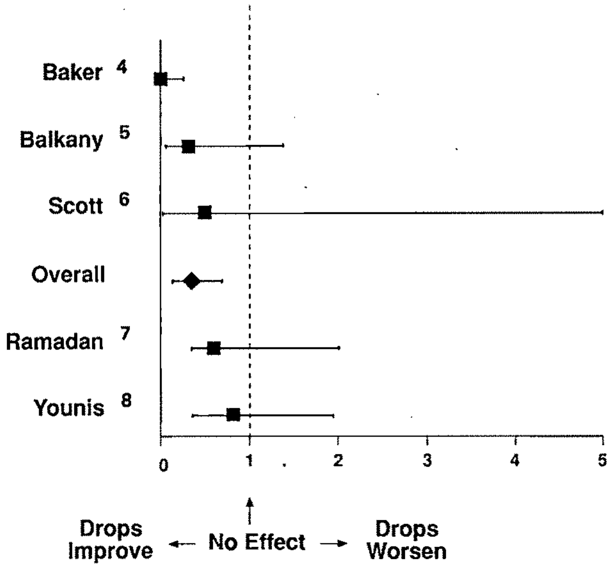
For each study, 2 × 2 contingency tables were constructed indicating the number of subjects or ears with or without otorrhea in columns and the number of subjects receiving prophylaxis or placebo in rows (see Table). Calculation of combined odds ratios (ORs) and 95% confidence intervals (CIs) was done by the method of Mantel-Haenszel[9] for combined 2 × 2 contingency tables from separate studies. Assume that each study enrolls N patients, n of whom receive prophylactic treatment and r of whom develop otorrhea. For each study, the Mantel-Haenszel procedure compares O, the observed number of subjects with otorrhea among the treated patients (or ears) with $E = nr/N$, the expected number of subjects with otorrhea. Assuming that the null hypothesis of no effect of the treatment is correct, the difference $O - E$ will differ randomly from zero and will have a variance given by $\mathrm{Var} = E(1 - n/N)(N - r)/(N - 1)$. If Sumdiff is the sum of the differences $O - E$ over all studies, and if Sumvar is the sum of the variances of these differences over all studies, it follows that an estimate of the combined ORs is given by $\mathrm{OR} = \exp[(\mathrm{Sumdiff} - 0.5)/\mathrm{Sumvar}]$. Also, SE, the standard error of the summed differences, is the square root of Sumvar, so that 95% confidence bounds on the combined odds ratio are given by $\exp[(\mathrm{Sumdiff} - 0.50)/\mathrm{Sumvar} \pm 1.95/\mathrm{SE}]$.

In the first 3 studies[4-6] the patient was the experimental unit (by-patient design), with PPO in either ear being scored as failure. In the other 2 studies[7,8] (by-ear design) 1 ear was treated and the other was used as a control. The by-ear design doubles the number of experimental units and creates problems in interpretation because of the interrelation between right and left ears.[10] Because of these differences, analyses were done for each subgroup (by-patient and by-ear). The Mantel-Haenszel method is based on independence of groups, which does not exist for the by-ear studies. Therefore, the meta-analysis was only applicable to the 3 by-patient studies. The Mantel-Haenszel method for estimating the within-studies confidence bounds on the ORs was used on studies 2 and 3, but for study 1 it could not be applied because of the zero cell. In study 1, the Woolf[11] statistic was used, with 0.5 added to each cell as suggested by Haldane.[12]

RESULTS

Analysis of Studies. Study 1[4] treated 56 children with gentamicin ophthalmic solution for 4 days, while the 46 children in the control group received no prophylactic therapy. There were no cases of otorrhea in the treated group, and 9 cases (19.6%) in the control group. Outcome was measured at 14 days.

Study 2[5] enrolled 34 children in the topical antimicrobial prophylaxis group, who received Cortisporin Otic Suspension (neomycin, polymyxin, hydrocorti-



Graphic display of odds ratios (ORs) for individual studies with 95% confidence interval and overall OR. OR of less than 1 indicates improvement from treatment; OR greater than 1 shows worsening. To be significant at p = .05 level, error bars must not cross no-effect line (OR = 1).

sone; Burroughs-Wellcome Co); 38 in an oral antibiotic (ampicillin) group; and 26 in a control group. The numbers of children with PPO in 1 or both ears during the first 4 postoperative weeks were 2 (6%), 5 (13%), and 5 (19%) for the 3 groups, respectively. The children in the oral antibiotic group were excluded from the meta-analysis (below), as they did not meet the entry criteria.

Study 3[6] randomly assigned 17 children to receive no prophylaxis, 17 to receive topical gentamicin ophthalmic drops in the operating room, and 18 to receive the same agent in the operating room and for 2 days postoperatively. Purulent postoperative otorrhea developed in 6 children (9 ears), ie, 3 children (4 ears) in the control group, 2 children (3 ears) in the intraoperative-only group, and 1 child (2 ears) in the group treated for 2 days (B. A. Scott, personal communication, December 1992). For the purpose of the meta-analysis (below), the 17 cases in the intraoperative treatment group were excluded because they did not meet the entry criteria for the meta-analysis.

Study 4[7] randomly assigned 60 children undergoing TT insertion to receive dexapolyspectran (a solution of polymyxin B sulfate, neomycin sulfate, sulfonamide, and hydrocortisone) in the right ear for 3 days postoperatively and no therapy for the left ear. Results were measured weekly for 4 weeks. Three children developed bilateral otorrhea; 2 additional children developed otorrhea in the treated ear, and 5 in the control ear (H. H. Ramadan, personal communication, December 1992).

Study 5[8] enrolled 200 children undergoing bilateral TT insertion to receive Cortisporin Otic Suspension in the right ear 3 times a day for 3 days postoperatively. Otorrhea in the first 3 weeks was scored as failure. Otorrhea occurred bilaterally in 23 children and unilaterally (in the control ear) in an additional 3 children (R. T. Younis, personal communication, December 1992).

The individual ORs and CIs for the studies grouped by experimental design are displayed in the Table.[4-8] For the 3 by-patient studies, the rate of otorrhea in either ear was 18.9% in the control children and 2.8% in the treated children. This represents an 85% reduction. The meta-analysis of the 3 by-patient studies showed a combined OR of 0.12 and a 95% CI of 0.04 to 0.37. For the 2 by-ear studies the otorrhea rate in the control ears was 13.1%, and in the treated ears it was 10.8% (see Figure).

DISCUSSION

Meta-analysis is an increasingly used technique to examine multiple studies in which conflicting results exist. The technique estimates an overall effect of the treatment under consideration. Combining the results from many subjects should permit a clearer view of overall trends that might not be apparent in smaller samples of subjects. Meta-analysis is a method of statistical inference, and the results should be interpreted cautiously. An OR of less than 1 indicates a reduction in PPO in the treated group, an OR of more than 1 indicates an increase in PPO in the treated group, and an OR of 1 indicates no difference between the groups.

In the present study, the OR for each study individually and for each of the 3 combined analyses was less than 1. However, in only 1 study was the upper bound of the 95% CI less than 1; an upper CI bound of 1 or greater indicates lack of significance at the p = .05 level. Although these analyses reveal a reduction in the incidence of PPO as a result of the use of topical antimicrobial prophylaxis, in only the by-patient studies did the combined 95% CI bounds exclude 1. The combined by-patient studies demonstrated an overall 85% reduction in unfavorable outcome, which most would consider to be clinically significant. However, given the heterogeneity of the experimental designs and the lack of a significant effect from the drops in the by-ear studies, we cannot be confident that the results of the meta-analysis are conclusive. Therefore, it would be prudent to consider the effect of other factors.

Giebink et al[13] noted a strong correlation between PPO and 1) positive middle ear cultures and 2) cases of mucoid middle ear effusion. Balkany et al[5] found the greatest incidence of otorrhea in the youngest children and in those with grossly purulent effusion. Scott and Strunk[6] also noted that the incidence of PPO was significantly higher in the cases of mucoid middle ear effusion as compared to those patients with serous or no effusion. Gates et al[14] found a higher incidence of PPO in children with culture-positive middle ear effusions (5.4%) than in those with sterile effusions (2.9%). Thus, these studies, which examined the effect of many factors on the incidence of otorrhea, suggest the operation of several risk factors for PPO. Logic would suggest that cases exhibiting these risk factors should receive the highest priority for prophylactic therapy.

Three variables not controlled in these 5 studies deserve mention: compliance in drug use, assurance of proper drug delivery technique, and type of agent and duration of use. It is not possible to determine if differences in outcome might have been due to variation in the technique of administration of the drug and compliance with instructions. The duration of topical prophylaxis and agents used varied among the studies: 1) gentamicin ophthalmic solution for 3 days, 2) Cortisporin Otic Suspension for 7 days, 3) gentamicin ophthalmic solution for 2 days, 4) Cortisporin Otic Suspension equivalent for 3 days, and 5) Cortisporin Otic Suspension for 3 days. Given the substantial difference in outcome between study 1 and the others, one could hypothesize that the difference was due to the difference in agent. It may be the case that topical gentamicin ophthalmic drops are truly more effective than the mixture of several antibiotics and hydrocortisone used in the other studies. However, it would be inappropriate to use the current analysis to test that hypothesis, and additional studies would be necessary. Given the known hazards of propylene glycol in the middle ear,[15] the formulation of the agent used in studies 1 and 3, which contains less propylene glycol, deserves further study.

A fourth variable, surgical technique, is a possible confounder. Disinfection of the external auditory canal has been investigated as a possible variable in the problem of PPO.[13,16] However, neither povidone-iodine nor 70% alcohol had a significant effect on PPO. The type of tube used depends on the type of effusion and the patient's diagnosis, which, in turn, are related to the rate of PPO. Therefore, we exclude technique as an important source of variation.

Bias. Combining studies in a meta-analysis usually involves mixing of nonparallel studies. In this case, mixing of studies with by-patient and by-ear designs creates substantial problems in interpretation because of the difference in denominators and heterogeneity. For example, the rate of PPO in the control groups in the by-ear studies differed substantially from that in the others, suggesting heterogeneity of populations. Because treatment effect in 1 ear is not independent of the treatment results in the second ear of the same subject, it is not appropriate to include by-ear studies in meta-analysis.

Although it may be the case that the inference resulting from the meta-analysis of the 3 by-patient studies — that there is a real effect of the agents — is warranted, it is our opinion that a convincing case for or against the use of topical antimicrobial prophylaxis against PPO cannot be made from the available evidence.

CONCLUSION

We believe the continued study of the use of topical antimicrobial prophylaxis following TT insertion is justified. We would argue that the studies should be limited to high-risk cases, that is, those children with mucoid or purulent effusion in whom the risk of ototoxicity is reduced by the presence of effusion and mucosal hyperplasia (which decrease absorption across the round window membrane). Using topical antimicrobial prophylaxis in middle ears that are free of effusion, in which the risk of PPO is low, may unnecessarily expose the cochlea to risk of ototoxicity. It should be stressed that ototoxicity is a theoretic risk because hearing loss from the use of topical antimicrobial therapy has not been confirmed in children. Nonetheless, the finding that agents placed in the middle ear readily cross the round window membrane to cause hair cell loss in experimental animals[17] suggests that caution should be exercised in this application in humans.

Additional studies should use the patient as the experimental unit, with randomization stratified on known risk factors (type of effusion, age, microbiology) to determine the effectiveness of topical antimicrobial prophylaxis for high-risk subgroups. However, until these are completed, we believe that selective use of topical antimicrobial prophylaxis following TT insertion in high-risk cases is not inappropriate.

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NASAL AND OTOLOGIC EFFECTS OF EXPERIMENTAL INFLUENZA A VIRUS INFECTION

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Past studies showed that experimental rhinovirus colds in adults resulted in eustachian tube dysfunction and abnormal middle ear pressures. In the present study, the symptoms and pathophysiologic findings accompanying experimental influenza viral infection were documented. A total of 33 healthy adult volunteers were intranasally challenged with an influenza A/Kawasaki/86 (H1N1) virus and cloistered over a 9-day postchallenge period to monitor for evidence of infection, signs and symptoms of illness, and the extent and frequency of pathophysiologic responses of the nose, eustachian tube, and middle ear. Results showed a protective effect of high (≥16) prechallenge specific hemagglutination-inhibition antibody titer on the rate of infection and the magnitude and extent of provoked symptoms and pathophysiologic findings. Infected subjects with low (<16) prechallenge serum antibody titers (n = 21) developed significant respiratory illness. These subjects also had objectively measurable increases in nasal secretion production, and decreased nasal patency and mucociliary clearance rates. More than 80% of the infected subjects developed eustachian tube dysfunction, and approximately 80% had middle ear underpressures of less than −100 mm H2O on study days 4 and 5. Five of 21 infected subjects with low prechallenge antibody titers had otoscopic evidence of otitis media with effusion. These results support a causal role for viral upper respiratory tract infection in the pathogenesis of otitis media, possibly mediated by the early development of eustachian tube dysfunction and abnormal middle ear pressure.

KEY WORDS — eustachian tube, influenza virus, nose, otitis media, upper respiratory tract infection.

INTRODUCTION

Otitis media is extremely common in the pediatric population.[1] Epidemiologic studies report that otitis media is a frequent complication of viral upper respiratory tract infection (URTI).[1-5] However, that viruses are seldom isolated from middle ear effusions suggests that local infection of the middle ear mucosa is not a prerequisite for disease expression.[6-9] A number of earlier studies reported a deterioration of eustachian tube function during a viral URTI.[10-12] In animal experiments, sustained tubal dysfunction results in the early development of middle ear underpressures followed by the accumulation of a middle ear effusion.[13] In chinchillas, dual infection of the nose and nasopharynx with *Streptococcus pneumoniae* bacteria and influenza virus results in middle ear underpressures and acute otitis media that is culture-positive for the challenge strain of bacteria.[14] These data suggest that viral URTI plays a causal role in the pathogenesis of otitis media. One possible mechanism linking these two disease entities involves virally mediated eustachian tube dysfunction resulting in a middle ear underpressure and subsequent otitis media with effusion due to either prolonged tubal obstruction or aspiration via the eustachian tube of nasopharyngeal bacteria.

Most past attempts to test hypothesized mechanisms by which viral URTI results in otologic disease have focused on experiments using the chinchilla or monkey as model systems.[14-16] However, that the eustachian tube function of the chinchilla has been shown to be quite different from that of humans suggests a limited ability to extend results from that model to humans.[17] While functionally similar to the human system with respect to the eustachian tube, the monkey does not present with clinical illness when infected with viruses commonly isolated during URTI episodes in humans.[18-20] To obviate these deficiencies, we initiated an experimental program involving intranasal viral challenge of adult volunteers. Previous work concentrated on rhinovirus, as this is the most common group of viruses associated with colds. Those studies showed that experimental rhinovirus infection results in significant increases in nasal symptoms and signs, impaired tubal function in a majority of subjects, and abnormal middle ear pressures in approximately 30% of infected subjects.[21,22] With that challenge system, the development of otitis

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media was a rare event (0% to 3%). More recently, in pilot experiments using small numbers of subjects we extended these observations to include infection with influenza and Coxsackie viruses.[23] The purpose of the present study was to document the response of the nose, eustachian tube, and middle ear to experimental influenza A viral infection in adults.

MATERIALS AND METHODS

Population. Subjects were recruited from the University of Pittsburgh and surrounding community. Applicants provided a general health history and were given otolaryngological and general physical examinations. Blood and urine specimens were collected. Blood samples were used for complete blood counts and assayed for a panel of hepatic enzymes and the presence of human immunodeficiency virus and hepatitis antibodies. Urines were submitted for urinalysis. Potential subjects were excluded if presenting with a history of nasal or otologic surgery, a history of cardiovascular or other serious disease, symptomatic upper respiratory infections within the last 30 days, concurrent nasal or otologic signs and symptoms, abnormal clinical profiles on urinalysis, complete blood count, or blood enzymes, or seropositivity for human immunodeficiency or hepatitis viruses. Thirty-three (20 men and 13 women) healthy adult subjects (20 to 39 years old) were enrolled after providing a written informed consent. The study was approved by the Human Rights Committee at the Children's Hospital of Pittsburgh.

Experimental Plan. The study was conducted during the month of November. Enrolled subjects reported to a hotel located within 8 km (5 miles) of the Children's Hospital of Pittsburgh during the evening (6:00 PM) of study day 0 and were cloistered in individual rooms for a 9-day period (study days 0 through 8). Viral inoculation was performed 24 hours after admission to the cloister site (6:00 PM, end study day 0).

On each study day, tissues and plastic bags of known weight were supplied at 6:00 PM and collected at 6:00 PM on the following day. A nasal lavage was performed during the morning hours, and symptoms were scored at approximately 12:00 noon. Three times per day, in evening, morning, and afternoon test sessions, oral temperatures were recorded and a number of physiologic functions were evaluated. These included evaluations of nasal patency by active posterior rhinomanometry, eustachian tube function by sonotubometry, and middle ear pressure by tympanometry. Additionally, during the afternoon hours, an otolaryngological examination was performed and the nasal clearance function was evaluated by a modification of the dyed saccharin test. Blood samples were collected on study day 0 and then again at 21 days postchallenge for assay of hemagglutination-inhibition antibodies to influenza A/Kawasaki/86 (H1N1) virus.

For the period of study, the subjects were asked to refrain from taking any over-the-counter or prescription medication with the exception of birth control pills. Additionally, alcohol consumption was not permitted. Acetaminophen was supplied and doses were recorded by study personnel as a rescue medication for those subjects requiring relief from incurred illness.

Viral Challenge. The challenge virus was a safety-tested clinical isolate of H1N1 supplied by the National Institute of Allergy and Infectious Disease (wild type, lot E-262). Viral challenge was performed with the subject sitting erect and the neck hyperextended. Then, 0.25 mL of inoculum per nostril was administered intranasally by pipet as coarse drops. The subject was instructed to perform a lateral head sway to ensure more complete distribution to the nasal mucosa, and to refrain from sneezing or nose-blowing for 30 minutes. For each subject, the total dose was estimated to be 10^7 median tissue culture infective dose.

Assessments of Infection. For viral culture, nasal lavages were performed on the morning of each study day by previously described methods.[21] An aliquot of the lavage fluid collected from each subject on the evening of study day 0 was submitted to the clinical laboratories at SmithKline Beecham for culture of common respiratory viruses in human diploid fibroblast, primary rhesus monkey kidney, primary African green monkey kidney, hep-2, and A549 cell lines (prechallenge culture).[24] At the termination of the 9-day period of study, samples of nasal lavage fluids for every subject on each study day were submitted to the virology laboratories at the University of Virginia for culture of influenza virus in triplicate monolayers of Madin-Darby canine kidney cells according to previously described methods.[25]

After collection of the convalescent serum samples, paired serum (day 0 and day 21) samples were sent to the virology laboratories at the University of Virginia and tested for hemagglutination-inhibition antibodies to H1N1 by standard methods.[26] Infection was defined as documented viral shedding on at least 1 postchallenge day of cloister or a fourfold increase in serum antibody titer between prechallenge and convalescent samples.

Subjective Assessments of Illness. At 12:00 noon on each study day, subjects were interviewed regarding the presence and extent of symptoms. Eight specific symptoms, including sneezing, nasal discharge, nasal congestion, malaise, headache, chilliness, sore throat, and cough, were rated by the subject on a four-point, 0 to 3 scale corresponding to none, mild, moderate, or severe. For analysis, three combined scores were calculated for each individual on each study day: nasal symptom score (sum of scores for sneezing, nasal discharge, and nasal congestion), throat symptom score (sum of scores for sore throat and cough), and systemic symptom score (sum of headache, chilliness, and malaise). Subjects were invited to list additional symptoms not specified on the scoring sheet. Presence of these symptoms was recorded by the study technician but not scored. On each study day, the subjects were asked if they believed that they had sufficient illness to qualify as having a cold or flu. These responses were recorded on the diary sheets. The subject was considered to be ill if he or she had reported a cold or flu on at least 2 days.

Objective Assessments of Illness. To provide a daily measure of secretion production, subjects were supplied with preweighed tissues at 6:00 PM on each study day. The subjects were instructed to expel all nasal secretions into these tissues and seal the expended tissues in plastic bags. These were collected a 6:00 PM on the following day and weighed. Secretion weights for each 24-hour period were determined by subtraction.

The degree of nasal congestion was measured three times per day via the technique of active posterior rhinomanometry and a computerized instrument developed in our laboratories and described previously.[21] The output variable, inspiratory work per liter (WPL), is a direct and linear measure of nasal congestion and has the units millijoules per liter.

The nasal mucociliary clearance function was measured by a previously described modification of the dyed saccharin technique.[21] Briefly, 15 μL of a test solution (Evans blue dye, saccharin) was placed bilaterally on the anterior aspect of the inferior turbinate. The time from application of the test solution to when the subject reports a sweet taste is defined as the clearance time, which is an inverse measure of clearance rate. This test was discontinued if a sweet taste was not reported by 30 minutes, in which case a clearance time of 30 minutes was assigned.

Otologic Assessments. All persons were examined daily by a resident otolaryngologist using pneumatic otoscopy. The appearance and mobility of the tympanic membrane and any evidence of abnormalities were recorded. Photographic documentation of the appearance of the tympanic membrane was made on days 4 and 8.

Middle ear pressures were measured bilaterally three times a day by means of a standard clinical instrument (Teledyne Impedance Screener). In the primary analysis, middle ears with pressures of less than or equal to −100 mm H_2O or middle ears with compliance values of less than 0.3 mL were considered to have abnormal pressures. For each study day, abnormal middle ear pressure was defined as abnormal pressures at any of the three test sessions conducted on that day. A person evidencing either unilateral or bilateral abnormal middle ear pressure was considered to have abnormal middle ear pressure on that study day.

Eustachian tube function was evaluated bilaterally three times per day by means of the method of sonotubometry and a computerized instrument developed in our laboratory and previously described.[27] For each study day, normal tubal function was defined as a positive test on at least one of the three test sessions conducted during that day. Since past studies in rhinovirus-infected subjects showed that the responses of the two ears were not independent, summary data are also presented for persons. Normal function for a person on each study day was defined as the presence of bilaterally normal eustachian tube function for that study day.

Statistical Methods. Because virologic assays were performed after completion of the cloister phase of the study, investigators and participants were blinded to the individual's prechallenge antibody titer as well as to the results regarding viral shedding or seroconversion. At the completion of the virologic assays, laboratory specific data were exchanged between the Children's Hospital of Pittsburgh and the University of Virginia. The primary analysis planned was a comparison of the response of the various measures to viral challenge in infected (I) and uninfected (UI) subjects. As described below, the infected group was further subdivided into two subgroups; a low-titer infected group (LT-I) consisting of subjects with prechallenge antibodies of less than 16, and a high-titer infected group (HT-I) consisting of subjects with antibody titers greater than or equal to 16.

For this analysis, secretion weight, WPL, clearance time, and the nasal, throat, and systemic symptom scores were considered to be continuous variables. The results for tests of eustachian tube function and middle ear pressure were treated as dichotomous, nominal variables (normal or abnormal).

To determine if a particular variable was responsive to influenza infection, the average daily values

MEAN AND STANDARD DEVIATION OF RESPONSE VARIABLES RECORDED FOR THREE COMPARISON GROUPS

Response Variable	*UI (N = 7)*	*HT-I (N = 5)*	*LT-I (N = 21)*	*LT-I vs UI*	*LT-I vs HT-I*
Nasal symptoms*	1.3 ± 6.5	3.6 ± 3.3	12.5 ± 6.8	$p < .05$	$p < .05$
Throat symptoms*	1.7 ± 2.0	2.2 ± 1.9	6.0 ± 5.8	$p < .05$	
Systemic symptoms*	1.6 ± 1.7	1.4 ± 1.0	7.5 ± 6.2	$p < .05$	$p < .05$
Mucus weight (g)	0.8 ± 1.6	5.7 ± 5.4	20.3 ± 23.8	$p < .05$	
Work per liter (mJ/L)	376 ± 217	361 ± 602	568 ± 599		
Clearance time (min)	7.9 ± 25.9	66.3 ± 38.4	38.7 ± 46.4	$p < .05$	

UI — uninfected; HT-I — infected, with high antibody titer; LT-I — infected, with low antibody titer.

*See text for scoring system.

of the continuous variables were calculated for each comparison group. The average values for each postchallenge test day were compared to their respective prechallenge average value by means of a Student's *t* test (two-tailed, evaluated at $p < .05$). For dichotomous variables, the daily frequencies of normal observations were calculated and the χ^2 statistic (with Yates correction for continuity) was used for these comparisons.

To evaluate the possibility of a differential response between comparison groups while avoiding the problem with multiple comparisons, a response variable was defined for each of the continuous variables based on the area under the curve. Specifically, for each individual the value of the function recorded on the baseline test day was subtracted from the respective values recorded for the individual on days 1 through 8. The value of the response variable for each individual was determined by summing his or her baseline adjusted values over the 8-day postchallenge period. For example, the response variable secretion weight was calculated for each individual by summing the weights recorded for the individual over study days 1 through 8 and then subtracting the product of 8 times the secretion weight recorded at day 0. For dichotomous variables, the response variable for each individual (or ear) was calculated as the sum of all normal observations recorded during the postchallenge cloister period. For example, if a given ear had positive eustachian tube function tests on 18 of the possible 23 test sessions conducted on days 1 through 8, the eustachian tube response variable for that ear was assigned a value of 18. The average values of these response variables were compared between groups by means of a Student's *t* test (one-tailed, evaluated at $p < .05$). The average values for the various response variables and the associated probability levels for the comparisons LT-I versus UI and HT-I are presented in the Table. In the descriptive data presentation, the convention mean ± 1 SD is used throughout.

RESULTS

Antibody Titers and Infection. Twenty-one of the 33 subjects had prechallenge antibody titers of less than 16, suggesting susceptibility to the challenge strain of virus. Additionally, 3 subjects had titers of 16, 7 had titers of 32, and 2 had titers of 64 prior to challenge. On study day 0, no respiratory viruses were isolated from the nasal lavage fluids of any study subject. After viral challenge, all of the 21 susceptible (100%, titers < 16) subjects were infected as indicated by viral shedding (n = 19, 90%) or a fourfold rise in antibody titer (n = 20, 95%). For that group, the average number of days on which the virus was isolated was 3.5 ± 2.0 and the average fold increase in antibody titer was 10.3 ± 5.1. Of the 12 subjects with prechallenge antibody titers greater than or equal to 16, 5 (42%) were infected as evidenced by viral shedding (n = 5, 42%) or a fourfold rise in titers (n = 2, 17%). For these 5 infected subjects, the average number of days on which the virus was isolated was 1.2 ± 0.4 and the average fold increase in antibody titer was 2.6 ± 1.2. By definition, the 7 uninfected subjects did not shed virus on any of the 8 postchallenge study days or seroconvert.

As stated in Materials and Methods, our original intent was to compare the various responses between the I and UI subjects. However, preliminary analysis of the data for I persons showed a clear distinction between the HT-I and LT-I groups with respect to most response measures (see Table). Consequently, in this presentation, the primary comparison groups are LT-I (n = 21), HT-I (n = 5), and UI (n = 7). Because of the relatively small sample sizes and heterogeneous responses of persons in the HT-I group, graphic data are presented only for the LT-I and UI groups. Statistical comparisons are limited to the LT-I versus the HT-I and UI groups.

Illness. Of the 21 LT-I subjects, 20 (95%) reported that they had a flu or cold on at least 2 postchallenge days. The average number of days with a reported flu or cold was 4.4 ± 1.8 days for that group. Additionally, 2 (40%) subjects in the HT-I and 2 (29%) in the UI group reported that they had a flu or cold on at least 2 postchallenge study days. One subject in the UI group reported a flu or cold on a single day of study (day 2). The average number of days with a reported flu or cold was 1.0 ± 1.4 days for the UI group and

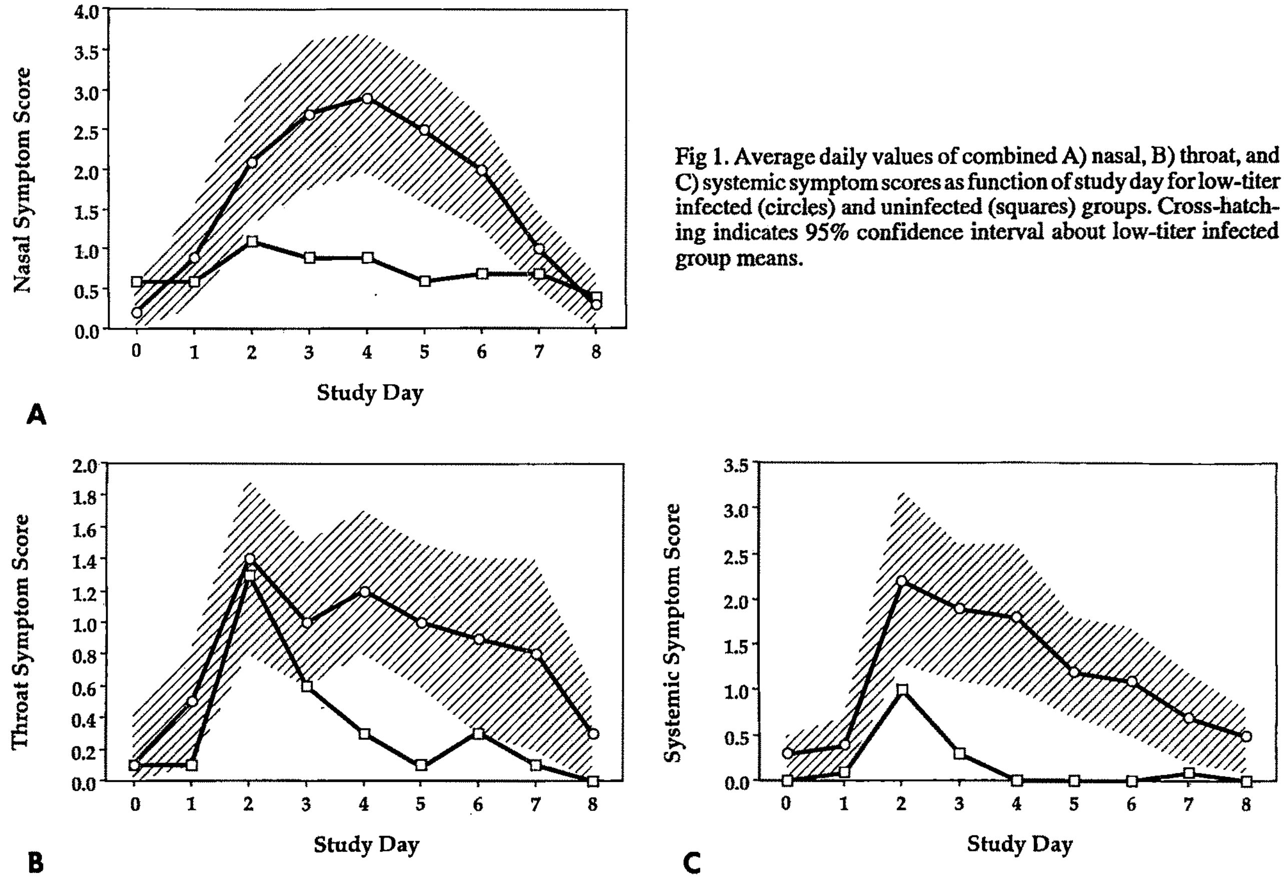


Fig 1. Average daily values of combined A) nasal, B) throat, and C) systemic symptom scores as function of study day for low-titer infected (circles) and uninfected (squares) groups. Cross-hatching indicates 95% confidence interval about low-titer infected group means.

1.0 ± 1.3 days for the HT-I group.

Symptoms. The averages for the three combined symptom scores recorded on day 0 were not different among groups. Figure 1 shows the daily average values of the nasal symptom score, throat symptom score, and systemic symptom score for the UI and LT-I groups. After virus challenge, the daily nasal symptom scores of the UI group were not significantly changed from that recorded at baseline. However, in the LT-I group, the nasal symptom score was significantly elevated by day 1 postchallenge, peaked on day 4, and returned to approximate baseline values by day 8. In contrast, the temporal patterns for the throat symptom score showed an early and significant increase, to peak on day 2 postchallenge in both groups. For the UI group, the throat symptom scores rapidly decreased to baseline values by day 5 postchallenge. Only the score recorded at day 2 was significantly different from the prechallenge value. For the LT-I group, significant increases in score relative to prechallenge values were maintained as late as day 6 postchallenge. The temporal patterns of change for the two groups in the systemic symptom score mirrored those of their respective throat symptoms in both magnitude and extent.

The average total nasal and systemic symptom scores were significantly greater in the LT-I group when compared to either the HT-I group or the UI group. For throat symptoms, the average total score of the LT-I was greater than that of both the HT-I and UI groups, although statistical significance was only achieved for the comparison with the UI group (see Table).

Fifteen additional symptoms were reported by subjects. As expected, the majority of symptoms were reported by persons in the LT-I group. Specifically, the average number of other symptoms reported by subjects was 0.6 ± 1.0, 0.4 ± 0.8, and 2.3 ± 2.1 for the UI, HT-I, and LT-I groups, respectively. Of the 21 subjects in the LT-I group, 52% reported body ache, 43% clogged ears, 24% lightheadedness, 29% earaches, 19% fever, and 14% sweating. The remaining symptoms varied in reported frequencies between 5% and 10% and included sore neck, dizziness, fatigue, itchy throat, shakes, migraine headache, chest pains, labored breathing, and sinus pain. One subject reported severe migraine headache, dizziness, and vertigo beginning on day 4 of the study and continuing throughout the period of cloister. Following release, the symptoms persisted and the subject was examined by an otologist and scheduled for clinical vestibular function testing on day 14 postchallenge. At that time, the symptoms were appre-

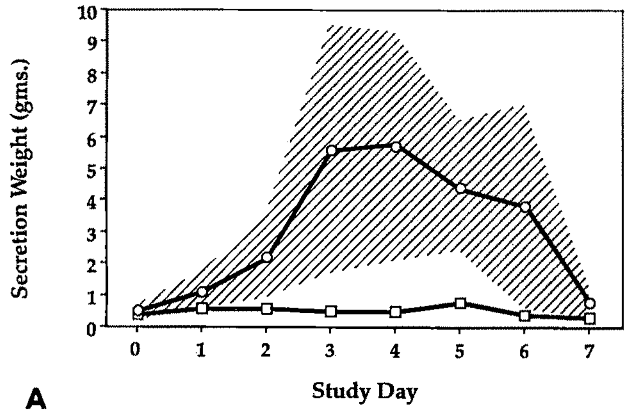
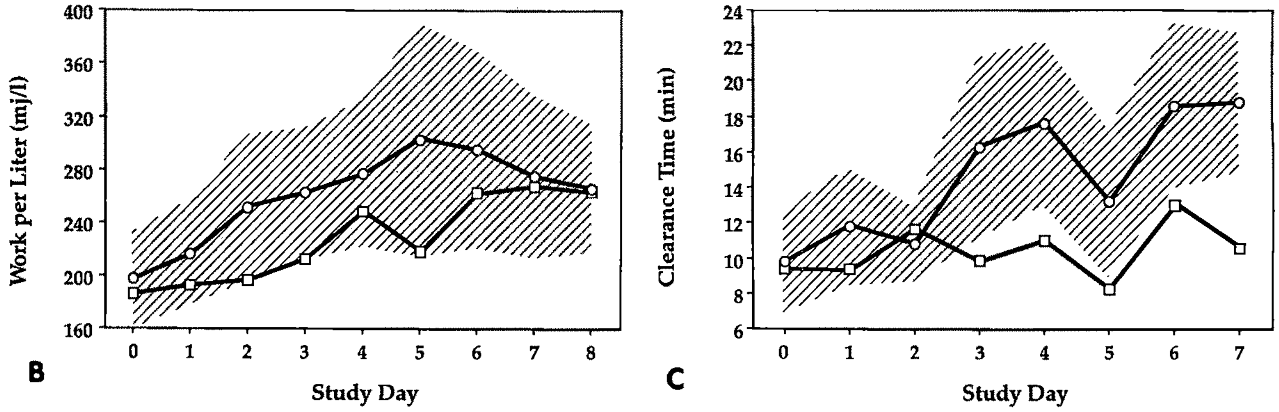


Fig 2. Average daily values of objective measures of A) nasal secretion, B) nasal congestion, and C) nasal clearance time for subjects in low-titer infected (circles) and uninfected (squares) groups. Cross-hatching indicates 95% confidence interval about low-titer infected group means.



ciably diminished, hearing was normal, and all measured responses to vestibular testing were within normal limits. By day 21 postchallenge and on presentation for postinfection convalescent phlebotomy, the subject reported complete resolution of the symptoms.

Body Temperatures. Oral temperatures were recorded three times per day on all study subjects during the period of cloister. Temperature elevations (≥37.7°C) were recorded on 1 (0.6%) of 161 determinations in the UI group, 27 (5.6%) of 483 determinations in the LT-I group, and 0 (0%) of 115 determinations in the HT-I group. Temperature elevations were recorded on at least one occasion in 12 LT-I subjects and on one occasion in 1 subject in the UI group. Four subjects in the LT-I group had a temperature of greater than 38°C on at least one occasion, and 3 of these subjects had temperatures greater than 38.5°C.

Secretion Weights. Secretion weights for the prechallenge study day were not significantly different among groups. Figure 2A shows the average daily secretion weights for the LT-I and UI groups. As with nasal symptoms, secretion weights were unchanged in the UI group following viral challenge. However, beginning on the day following challenge and extending for 6 days, the average daily secretion weights in the LT-I group were significantly greater than that recorded on the day prior to viral challenge. The temporal pattern of change in secretion weights mirrored that of nasal symptoms, with a peak 3 to 4 days postchallenge and a decrease to approximate the prechallenge average daily secretion weight by study day 7.

The average baseline adjusted total secretion weight for the postchallenge study days was greatest in the LT-I group, intermediate in the HT-I group, and least in the UI group. The average value for the LT-I group was significantly greater than that of the UI group (see Table).

Nasal Patency. Two subjects, 1 in the LT-I group and 1 in the UI group, could not reproducibly perform active posterior rhinomanometry. Consequently, data for those 2 subjects were not available for analysis. Prior to challenge, the average values of the WPL for the groups were not significantly different. Figure 2B shows the average daily WPL for the LT-I and the UI groups. The LT-I group showed a progressive increase in WPL to peak at day 5 and remain elevated for the duration of the follow-up. The daily values were significantly different from baseline on days 3 through 8. The UI group also showed a relative increase in WPL beginning on study day 5 and continu-

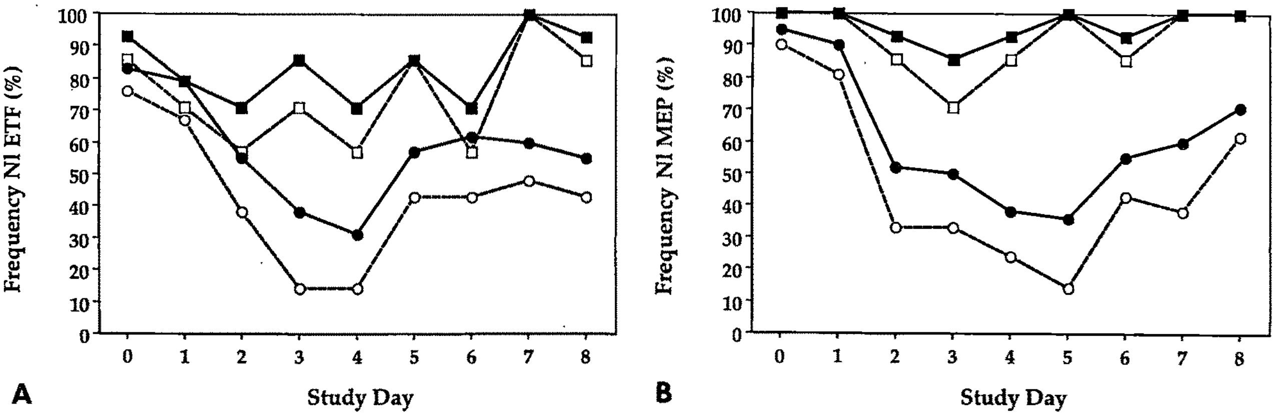


Fig 3. Average daily frequencies of persons (open symbols) and ears (closed symbols) with A) good or normal (Nl) eustachian tube function (ETF) and B) normal (Nl) middle ear pressure (MEP) for subjects in low-titer infected (circles) and uninfected (squares) groups.

ing for the duration of the follow-up period. By days 7 and 8, the average values of WPL were almost identical for the UI and LT-I groups. Because of the small sample size in the UI group, the average WPL was not significantly different from baseline on any study day.

The average total increases in WPL for the three groups are presented in the Table. While this value was greater in the LT-I group when compared to that of both the HT-I and UI groups, the between-group differences were not statistically significant.

Mucociliary Clearance Time. The nasal clearance time is an inverse measure of clearance rate. Four subjects, 3 in the LT-I group and 1 in the HT-I group, did not taste the test solution on the baseline test day or at any time during the study. These 4 subjects were not included in the data analysis. Prior to challenge, the clearance times were not significantly different between groups. In the LT-I group, prolongations in clearance time were observed beginning on day 3 and extending for the duration of follow-up. For that group, data recorded on days 3, 4, 6, and 7 were significantly different from the baseline value. In contrast, the average daily clearance times for the UI group varied about the prechallenge baseline, with no significant differences observed on any study day (Fig 2C).

The average total increases in clearance times for the three groups are presented in the Table. Here, the greatest increase was observed in the HT-I group, followed by the LT-I group and the UI group. The difference between the LT-I and UI groups was statistically significant.

Eustachian Tube Function. For the UI and LT-I groups, the daily frequencies of normal eustachian tube function are presented for both ears and persons in Fig 3A. On the prechallenge test day, the frequency of ears with normal eustachian tube function was not different for the UI (93%) and LT-I groups. After challenge, the frequency of ears with normal eustachian tube function was significantly decreased in the LT-I group, to 31% by 4 days postchallenge, with evidence of partial recovery by 5 days postchallenge. A clear pattern of change in this measure was not observed over the study period for the UI group, in which frequencies of normal function varied between 71% and 100%. A similar response pattern was observed for the two groups with individuals considered as the experimental unit. Prior to challenge, 86% of the individuals in the UI group and 76% of the individuals in the LT-I group had good tubal function. For the LT-I group, this frequency was significantly decreased, to 14% on days 3 and 4 postchallenge, with partial recovery of function by day 5. As with ears, the frequency of persons in the UI group with normal tubal function was not significantly decreased following challenge.

The averages of the numbers of abnormal observations for eustachian tube function testings were significantly different between the UI and LT-I groups for both ears and persons. Specifically, 7.8 ± 7.1, 10.5 ± 5.5, and 15.4 ± 5.5 of the observations for ears and 10.6 ± 7.9, 15.0 ± 4.6, and 18.0 ± 3.9 of the observations for persons were abnormal in the UI, LT-I, and HT-I groups, respectively.

Middle Ear Pressure. On baseline testing, between 90% and 100% of the ears and persons had middle ear pressures greater than −100 mm H2O. By this definition of normal middle ear pressure, the frequency of ears with normal pressures in the LT-I group decreased to approximately 40% by day 5, with a shallow recovery by day 8 (Fig 3B). On day 3, the frequency of ears with normal middle ear pressures had decreased to approximately 90% in the UI group, but normal pressures were restored in all ears by day

5. The frequencies of persons in both groups with abnormal middle ear pressures closely tracked those for ears. In the LT-I group, the frequencies of normal middle ear pressures were significantly different from baseline on days 2 through 7.

As discussed, the definition of abnormal observations included ears with either middle ear pressures less than –99 mm H2O or those with undefined pressures resulting from abnormally low tympanometric compliance (<0.3 mL, ie, flat tympanograms). Considering only middle ear pressures less than –99 mm H2O as abnormal, the average numbers of postinoculation abnormal observations for the ears in the UI, HT-I, and LT-I groups were 0.4 ± 1.3, 1.5 ± 2.0, and 5.5 ± 5.3, respectively. The between-group differences in this measure were statistically significant. No postinoculation observations were characterized by abnormally low compliances in the UI or HT-I groups. However, for the LT-I group a total of 37 observations (3.8%) in 10 ears of 8 subjects were characterized by low compliances. Of these, 7 ears had 2 or fewer abnormal observations, while 3 ears of 2 subjects had at least 7 abnormal observations. The subject with multiple observations (7 right ear and 14 left ear) of bilaterally abnormal compliance also reported migraine headache, dizziness, and vertigo as described above.

As reported for experimental rhinovirus infections, abnormal positive pressures were observed in this study. Also, in the earlier rhinovirus studies a different criterion for abnormal middle ear pressures was used. To allow for comparisons with those results, a second definition of normal middle ear pressures was used for between-group comparisons such that normal pressure was defined as pressures greater than or equal to –50 mm H2O and less than or equal to 20 mm H2O. This revised criterion did not change the rank ordering of the three groups with respect to frequency of abnormal observations. Specifically, the averages of the number of abnormal observations for ears were 3.1 ± 4.0, 5.2 ± 4.2, and 13.5 ± 5.7 for the UI, HT-I, and LT-I groups, respectively.

Otoscopic Findings. Prior to challenge, all subjects presented with normal otoscopic findings. By 3 days postchallenge, 17 of 26 (65%) I subjects had at least one ear with one or more of the following physical findings: tympanic membrane erythema, decreased mobility or retraction of the tympanic membrane, or scant mucus on the medial surface of the tympanic membrane. By day 4, 2 of these subjects had developed unilateral serous effusions as evidenced by an air-fluid level. On the following day, 23 (88%) of the I subjects had otoscopic abnormalities and 3 had evidence of unilateral serous effusion. These abnormalities had begun to resolve by study day 8, at which time 18 (69%) of the I subjects had otoscopic abnormalities. Over the course of the period of study, 6 ears of 5 subjects developed otoscopic evidence of otitis media with effusion. The 1 person evidencing bilateral otitis media also reported migraine headache, dizziness, and vertigo as described above. All 5 subjects with documented otitis media were in the LT-I group.

DISCUSSION

The results of this study document the temporal pattern of disease expression following influenza A viral infection. For most symptoms, onset occurs within 2 days of infectious exposure, with a peak symptom load on day 3 or 4 and a resolution by day 8. Symptoms were not confined to the nose, but extended to the throat and middle ear. Indeed, symptoms of sore throat were reported quite early in the course of infection, heralding the development of the nasal symptoms of congestion, rhinorrhea, and sneezing. Systemic symptoms including malaise, fatigue, chills, sweating, headache, body ache, and fever were also reported as early events that diminished in intensity with increasing time postexposure. The majority of these subjects (95%) judged the symptoms to be of sufficient intensity to qualify as a cold or flu.

The changes in an objective measure of nasal secretion tracked well the temporal course of nasal symptoms. However, the objective measure of congestion, nasal WPL, increased in parallel with symptoms but remained elevated throughout the period of follow-up despite the decline in average symptom score. Other objective tests showed a decreased nasal mucociliary clearance rate that extended for the duration of the 8-day follow-up, and the development of eustachian tube dysfunction and significant middle ear underpressures that followed a temporal pattern similar to that of the nasal symptoms. Otoscopic examination documented signs of middle ear abnormalities with frank expression of otitis media with effusion in 5 subjects beginning as early as 4 days after viral exposure. One subject developed symptoms of dizziness and vertigo suggestive of transient inner ear involvement. All signs and symptoms of illness had resolved by 21 days postinoculation.

In agreement with previous reports,[28] the results of this study document the protective effect afforded by high serum antibody titers. Specifically, the infection rate was less in subjects with prechallenge antibody titers of greater than or equal to 16 (42%) when compared to those with lesser antibody titers (100%). Subjects not evidencing infection did not develop significant nasal symptoms or signs. They did report a transient increase in throat and systemic symptoms, with expression limited to the second day after viral challenge. Also, 2 of the 7 (29%) UI subjects believed that they had a cold or flu. These reported symptoms may represent a placebo effect associated with the postexposure expectations of the subjects with respect to illness. Alternatively, the development of these symptoms may reflect the initiation of an infectious process with a rapid termination consequent to an early activation of the immune response. Attendant pathophysiologic signs documented in the LT-I group, including reduced mucociliary clearance rates, eustachian tube dysfunction, and the development of abnormal middle ear pressures, were not expressed in the UI subjects. Moreover, with the exception of clearance rate, the symptom load, frequency of persons who believed that they had a cold or flu, and the frequency and extent of nasal and otologic pathophysiologic findings were less in the HT-I subjects when compared to the LT-I subjects. Also, otitis media with effusion as a complication of viral infection was limited to those subjects with low antibody titers. These results suggest that high serum antibody titers can protect from infection, minimize the local expression of disease, and reduce complications in anatomically contiguous structures.

By means of similar methods, the nasal and otologic manifestations of experimental rhinovirus infection in adults were previously described.[21,22] In those studies, the spectrum of symptoms, signs, and pathophysiologic findings was similar to that described for influenza virus infection in this study. However, the severity of systemic symptoms reported in this study was greater than that reported for rhinovirus infections. Also, the frequency and magnitude of otologic complications were greater for adult subjects infected with influenza. Specifically, abnormal middle ear pressures were reported in approximately 30% of rhinovirus-infected subjects but in more than 80% of the influenza-infected subjects. For experimental rhinovirus infection, the development of otitis media with effusion was a rare event, with frequencies varying between 0% and 3%, as compared to 19% of the influenza-infected subjects in this study (C. A. Buchman, unpublished data). These observations are supported by epidemiologic studies that report otitis media as a more frequent complication of concurrent URTI caused by influenza when compared to those infections in which rhinovirus was isolated.[29]

In children, otitis media is the single most frequent reason for presentation to physicians for nonwell visits.[1] Epidemiologic studies document that more than 50% of new episodes of otitis media are diagnosed immediately following or concurrent with a viral URTI.[2,3] A causal relationship between the disease entities is supported by experimental studies in animals showing the development of otitis media as a complication of influenza viral infection[14-16] and by the results of a recent study in children showing a significantly lesser incidence of acute otitis media in infants and children immunized with influenza viral vaccine when compared to nonimmunized controls during a seasonal epidemic.[30] The protection efficacy of an attenuated influenza vaccine with regard to otitis media was also demonstrated in chinchilla experiments.[31] Thus, a large number of new episodes of otitis media with effusion are potentially preventable by interventions that reduce the frequency of viral URTIs or interfere with the process by which these infections are expressed as an otologic complication.

Regarding the latter, a variety of evidence supports a causal role for virally initiated disruption of normal eustachian tube function in the pathogenesis of otitis media during a URTI. In support of this hypothesis, tubal dysfunction has been documented in children and adults with natural viral URTIs,[10-12] in adults experimentally infected with respiratory viruses,[21,22] and in animals infected with influenza virus.[14,15,32] Sustained tubal dysfunction results in significant middle ear underpressures and consequent otitis media with effusion.[13] These observations and the results of this study show that viral URTIs can provoke eustachian tube obstruction, which in turn results in middle ear underpressures and otitis media with effusion.

Indeed, recent studies suggested potential mechanisms whereby a viral URTI could cause disruption of normal eustachian tube function. For instance, URTIs have been demonstrated to result in the local release of a variety of inflammatory substances,[33] some of which were shown to provoke tubal dysfunction when applied locally to the normal nasal mucosa.[34] In the chinchilla, damage to the eustachian tube mucosa was evident following nasal infection with influenza, possibly contributing to the tubal dysfunction.[14]

The major events predicted by this mechanism — eustachian tube dysfunction, middle ear underpressures, and otitis media with effusion — were realized in the present cohort of subjects infected with influenza A virus. Moreover, the observed onset times and frequencies of responders for the component events are consistent with a causal mechanism. However, additional studies with large numbers of subjects and employing targeted interventions are necessary to determine if this event cascade provides an accurate mechanistic description of the pathogenesis of otitis media in patients with viral URTIs. Validation of this mechanism would suggest new approaches to the prevention of otitis media by defining both early targets for prophylactic intervention and the time window available for initiating such intervention. Also, in studies of prophylactic efficacy, the measurements of eustachian tube function and middle ear pressure could serve as surrogate outcomes for the frank development of otitis media. This would greatly decrease the sample sizes required for such studies, given the low frequency of otitis media in adults with viral URTIs. There, prevention of the more frequently observed preconditions (ie, eustachian tube obstruction, abnormal middle ear pressures) would translate into prophylactic efficacy regarding expression of these preconditions as otitis media.

The results of this and earlier experiments[21-23] show that experimental challenge of adults with respiratory viruses can be used as a model to evaluate hypotheses regarding the mechanism by which viral URTIs cause otitis media with effusion. In addition to the specific mechanism advanced in this discussion, future studies could evaluate the contribution of other factors suggested to mediate the relationship. These include altered bacterial adherence,[35-37] modulation of the immune response,[38,39] interference with antimicrobial efficacy,[40] and depressed ciliary clearance of effusion secondary to a viral URTI. This knowledge would greatly aid the development of rational strategies for prevention of otitis media as a complication of URTI.

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MANAGEMENT OF THE TINNITUS PATIENT

Management of the Tinnitus Patient will be held in Iowa City, Iowa, October 14-15, 1994. For information, contact Regina Tisor, Workshop Secretariat; telephone (319) 356-2471; fax (319) 353-6739.

SOFTWARE REVIEW

SUPERSCOPE II FOR USE IN ELECTROPHYSIOLOGIC RECORDINGS

Company: GW Instruments, Inc, 35 Medford Street, Somerville, MA 02143; phone (617) 625-4096.

Hardware requirements: Macintosh computer, II series, Centris 650, or Quadra series recommended (68020 microprocessor or newer, with Nubus slot); A/D converter board (eg, MacADIOS II, also made by GW Instruments).

Software requirements: System 6.0.7+, but System 7 is recommended. Four megabytes of RAM is minimum; 8 megabytes is recommended.

Price: $1,490.

With an analog to digital converter, SuperScope II software turns the Macintosh computer into a data collection and processing device. It becomes a compelling alternative to traditional oscilloscopes, chart recorders, and spectrum analyzers. In our laboratory, it more than replaces the traditional oscilloscope for several reasons. First, it takes advantage of the computer's memory by storing any or all of the wave data in digital format. Second, there is a full range of software tools to help with data analysis. Third, the program features both graphical and spreadsheet capabilities for waveform editing. All of these tools are provided in the visually pleasing, easy-to-use Macintosh format.

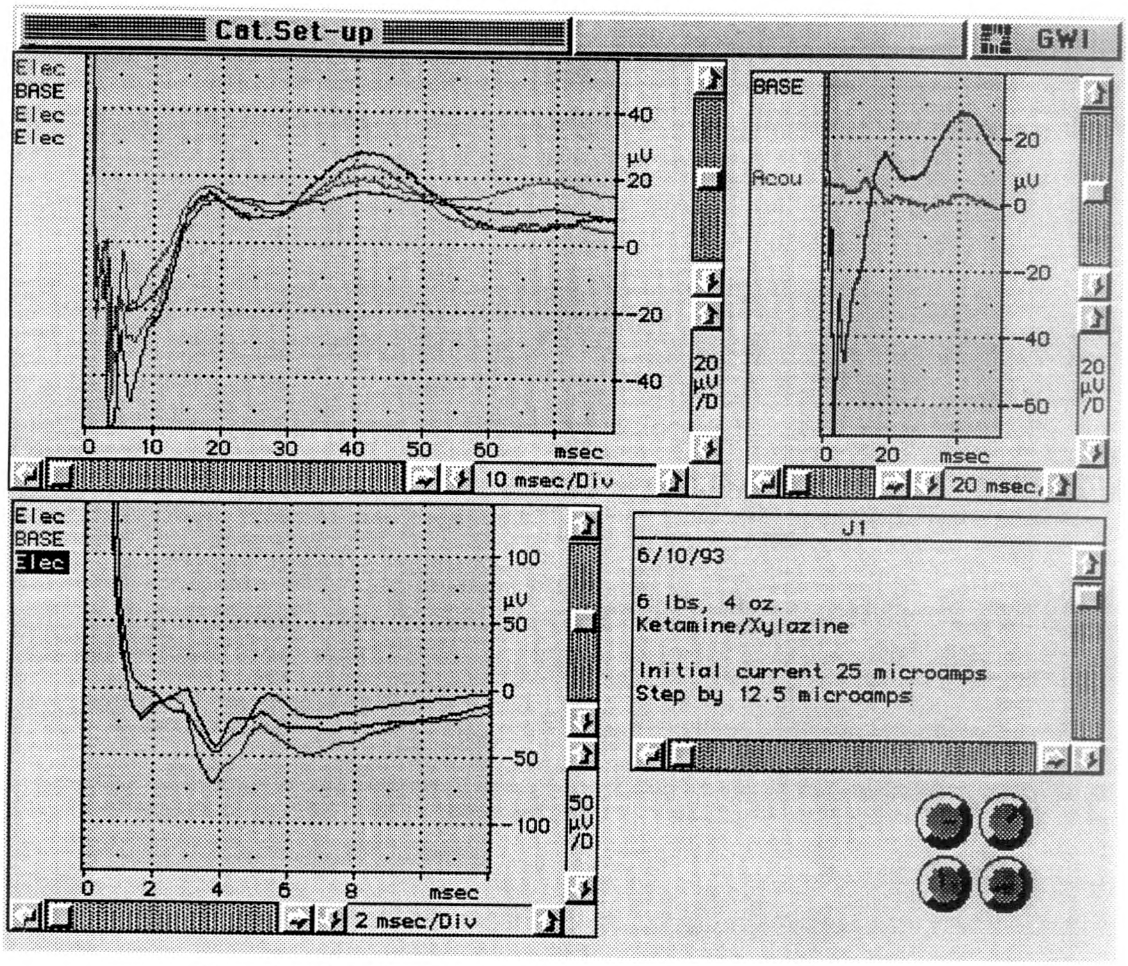
In our application, we use the original SuperScope and the new SuperScope II to record acoustic N_1 potentials, both acoustically and electrically induced middle latency responses in the feline cortex, whole nerve action potentials from the recurrent laryngeal nerve, and electromyograms of facial and laryngeal musculature. Our equipment includes a voltage generator used to stimulate the experimental animal, and standard equipment to amplify the electrophysiologic signal. In order to convert the analog signal to the digital format used by the Macintosh, we use an analog to digital converter board (MacADIOS II, also manufactured by GW Instruments). Our sampling periods have varied from 20 to 200 microseconds. The capacity to record eight or more channels of data simultaneously is present. In our application, however, very fast sampling rates are required that limit the number of available channels.

The user begins a session by tailoring the program to the experiment. Parameters for data recording, data presentation, and data storage are specified. For example, when measuring auditory cortex responses, we set SuperScope II for a trigger signal from channel 1, and the physiologic signal (generated via a cochlear implant) on channel 2, sampled every 200 microseconds. The updated signal-averaged data are continuously displayed, and the final signal-averaged data (after a specified number of experimental runs) are stored. Of course, the user also has the option to store every single response.

The user can also design the appearance of the screen (see Figure). In addition to displaying "waves" (acquired or computed data) in one or more "oscilloscope" panels, alternate windows can be opened to display other items. One example would be a journal. Journals can be run with SuperScope II's own word processing unit, avoiding the use of standard laboratory notebooks. Windows can also be used to display items such as spread sheets, coordinates of the mouse, or a macro editor. Items such as user-defined buttons or gauges can be added.

To run the experiment, the user then implements a macro, which is a list of commands that the computer serially executes. Although this sounds dangerously complicated, we have found this "programming" procedure quite straightforward; in fact, no true programming skills are required. Building on the standard Macintosh interface, SuperScope II provides a series of menus and windows for specifying each step of the process. The potential time savings makes the effort of becoming familiar with the macro system well worthwhile.

Once data are collected, the user can print in chart or spread sheet format, store a permanent record, or edit the raw data. SuperScope II stores data as "docu-



6/10/93

6 lbs, 4 oz.
Ketamine/Xylazine

Initial current 25 microamps
Step by 12.5 microamps

Example of SuperScope II screen, with user-defined windows and buttons.

ments," a term familiar to Macintosh users. Just as word processors produce text documents, SuperScope produces "wave" documents. As for editing, SuperScope II, like the original SuperScope, contains waveform analysis capability and a statistical package. In addition, the user can write user-specific macros that quickly simplify data analysis. For example, we used these features to break down our complicated electroencephalographic wave data into three pairs of coordinates corresponding to the major peaks and troughs.

Many improvements are made on the original SuperScope program. First, the macro ("tasks") feature is new. This allows more specific tailoring and efficiency in experimental design. Second, SuperScope II gives the user the ability to change the menu bars, deleting superfluous commands, thus simplifying experimental procedures in multiple user environments. Third, the appearance of screen windows can be customized, including user-defined buttons, knobs, dial gauges, and numeric readouts. Fourth, in order to provide more efficient access to documents in the Macintosh's sometimes burdensome system of folders, SuperScope II makes use of user-defined "data pipes" that help speed access to frequently used folders. In general, SuperScope II is more flexible and is easily suited for multiple-user environments.

Of note, for those interested in speech and sound analysis, GW Instruments offers an upgrade from SuperScope II to their SoundScope product.

We have found this oscilloscope simulation package to be extremely satisfactory. In our experiments measuring auditory function (by N1 recordings, auditory brain stem response, and middle latency response) and whole nerve action potentials, and in obtaining electromyographic activity of the facial and laryngeal musculature, no limitations were imposed by this software. The documentation accompanying the software and hardware is superior. It is easily understood, and the tutorials are well designed. In addition, GW Instruments has provided excellent technical support, with highly competent personnel handling our telephone inquiries.

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IMAGING CASE STUDY OF THE MONTH

ABNORMAL ENHANCEMENT IN THE REGION OF THE VESTIBULAR AQUEDUCT ON MAGNETIC RESONANCE IMAGING IN A CEREBELLOPONTINE ANGLE MENINGIOMA

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The vestibular aqueduct and the endolymphatic duct and sac have been described by high-resolution computed tomography (CT) and high-resolution three-dimensional magnetic resonance imaging (MRI).[1,2] We describe what we believe to be abnormal enhancement of the endolymphatic duct and sac with surrounding cerebellopontine angle (CPA) meningioma. To our knowledge, abnormal enhancement of these structures has never been described in cases of meningioma.

CASE REPORT

A 40-year-old woman was evaluated for a 4-month history of occasional ringing in her right ear and an intermittent, strange sense of motion when she was at rest. She denied vertigo, hearing loss, headache, nausea, vomiting, diplopia, weakness, or sensory loss. Her physical examination yielded normal findings, and she was neurologically intact. The results of her laboratory studies were unremarkable.

An MRI examination performed on a 1.5-T (GE, Milwaukee, Wis) superconductive scanner, using a slice thickness of 4 mm, showed a mass in the right CPA and internal auditory canal (IAC). This mass was isointense on precontrast T1-weighted images (Fig 1A) and showed intense enhancement with a

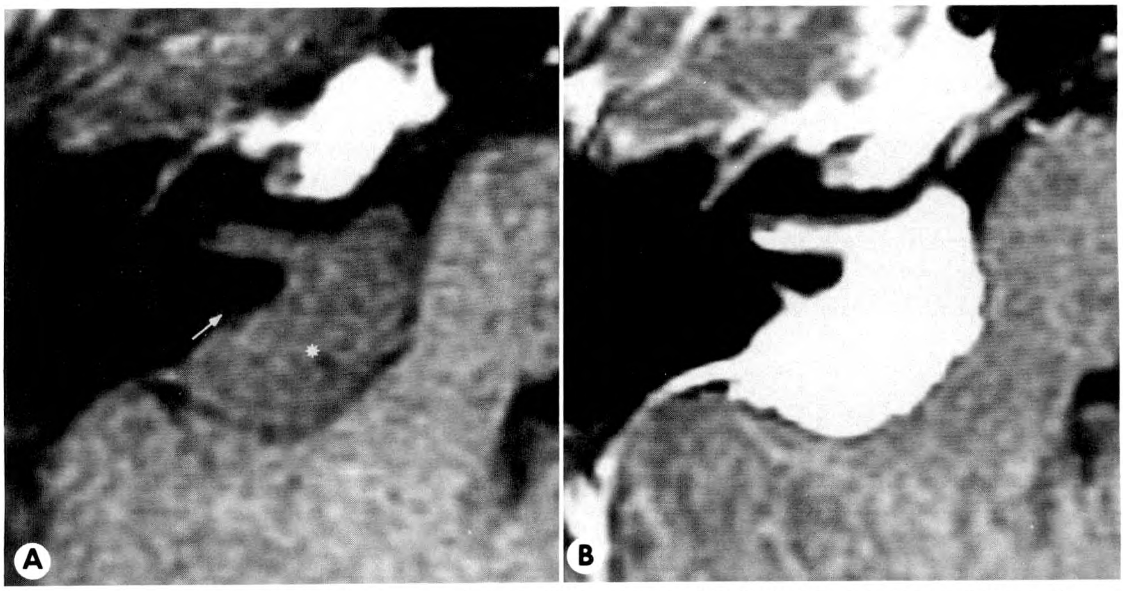

Fig 1. Axial T1-weighted magnetic resonance images. A) Precontrast (TR/TE 400/12), showing cerebellopontine angle mass (asterisk) with involvement of right internal auditory canal (IAC). Mass was relatively isointense to brain parenchyma on both T1- and T2-weighted (not shown) images. Note hypointense comma-shaped structure (arrow) posterior to right IAC, anatomically corresponding to vestibular aqueduct. B) Postcontrast (400/12), corresponding to A, showing marked enhancement of mass, IAC, and comma-shaped structure. (Gadolinium–diethylenetriamine pentaacetic acid, dose 0.1 mmol/kg as recommended by manufacturer.)

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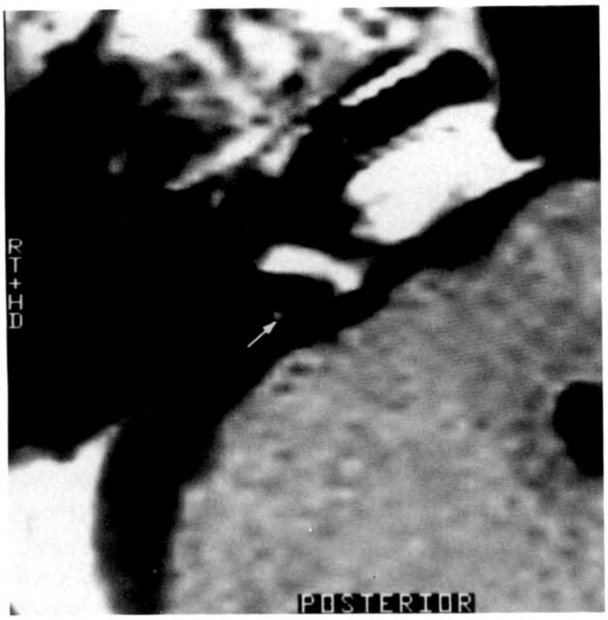

Fig 2. Follow-up postcontrast axial T1-weighted magnetic resonance image (TR/TE 450/20) at 4 months showing postoperative changes in internal auditory canal and persistent enhancement of previously noted comma-shaped structure (arrow).

dural tail sign on postcontrast studies, consistent with an acoustic meningioma. A small, enhancing, comma-shaped structure was seen arising from the anterolateral aspect of the mass and was thought, on the basis of its anatomic location, to represent enhancement of vestibular aqueduct contents and/or endolymphatic sac surrounded by dura and/or periosteum (Fig 1B).

At operation, through a right suboccipital retromastoid approach, the petrous ridge mass was dissected away from both the seventh and eighth cranial nerves, and the tumor was mobilized from the porus acusticus. A small portion of the posterior wall of the porus acusticus was removed. This allowed for the removal of the tumor, which had infiltrated the porus.

On pathologic examination, the mass was confirmed to be a meningioma. The patient had an uneventful postoperative course and was free of neurologic deficits. An MRI examination obtained 4 months later on a 0.5-T (Picker International, Highland Heights, Ohio) scanner showed no evidence of a tumor mass in the right CPA (Fig 2). A small area of enhancement within the IAC may represent reactive changes in the richly supplied connective tissue.[3] A significant decrease in enhancement in the region of the vestibular aqueduct was demonstrated in the follow-up examination.

DISCUSSION

The vestibular aqueduct is a tiny bony canal that courses posterolaterally through the posterior aspect of the petrous bone; distally, the aqueduct ends near the sigmoid sinus, best seen on high-resolution CT.[2,4] The vestibular aqueduct contains the endolymphatic duct and specialized, loosely textured connective tissue surrounding the duct, contiguous with the periosteum of the vestibular aqueduct. Only a small part of the proximal portion of the endolymphatic sac lies within the distal vestibular duct; the much larger portion extends out between the periosteal portion of the dura and the dura proper, with extremely vascular tissue surrounding it.[4] Although enhancement of the endolymphatic sac has been reported in patients with Meniere's disease,[5] enhancement in the region of the sac or within the vestibular aqueduct has not been described.

Our case showed an anterolateral, comma-shaped, enhancing structure thought to correspond to the region of the vestibular aqueduct because of its location. This can be explained by reactive and infiltrative changes in the dura and periosteum surrounding the endolymphatic sac or reactive changes in the specialized connective tissue surrounding the endolymphatic duct within the vestibular aqueduct. Part of this connective tissue reaction involves vascular plethora and inflammatory changes, both of which would be readily demonstrated on contrast-enhanced MRI. The reduction in pathologic contrast enhancement after surgery would further support this hypothesis. On the other hand, the meningioma could actually have invaded the periosteum and/or dura surrounding the endolymphatic sac. The remnant of abnormal contrast enhancement in the fovea of the vestibular aqueduct would also be consistent with our MRI findings.

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IMAGING CASE STUDY OF THE MONTH

FIBROUS DYSPLASIA OF THE TEMPORAL BONE

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CLINICAL FEATURES

A 62-year-old woman presented with transient attacks of giddiness of 10 years' duration. She described it as a feeling of blacking out that occurred several times a day. She had been deaf on the right side since childhood, and since the age of 5 had had an unexplained facial palsy on the same side. Clinical examination, apart from a right lower motor neuron type of facial paralysis, was unremarkable. A pure tone audiogram demonstrated that there was no hearing on the right side and about 40 dB sensorineural deafness on the left side. A computed tomography (CT) scan was performed to detect the underlying temporal bone disorder (see Figure).

RADIOGRAPHIC FINDINGS

There was an expanding lytic lesion of the medial aspect of the right petrous temporal bone, extending into the clivus. The bone had a ground-glass texture, and there was no evidence of bone destruction. The internal auditory canal was distorted with abnormal posterosuperior displacement of the meatus, which was narrowed. The inner ear and middle ear structures were preserved, and the mastoid air cells were well pneumatized. All appearances were characteristic of fibrous dysplasia.

DISCUSSION

Fibrous dysplasia as a specific disease entity was recognized by McCune and Bruch[1] in 1937. The most comprehensive review of fibrous dysplasia affecting the temporal bone, including the presenting and radiographic features and differential diagnosis, was given by Nager et al.[2] Fibrous dysplasia is a fairly localized disorder of bone that may be monostotic or polyostotic and is characterized by abnormal proliferation of fibrous tissue with normal or immature bone. Skeletal deformities and aberrations occur, and occasionally certain endocrinopathies and abnormal skin pigmentation form part of the clinical picture. Although the craniofacial skeleton is a common site, the temporal bone rarely becomes involved.

Pathologically, fibrous dysplasia appears as a poorly localized lesion replacing normal bone. The consistency is variable, and it may appear white to red, depending on its vascularity. The bony cortex may be expanded and thinned, but usually remains intact, maintaining a smooth contour. There is no true capsule, and the margin between the normal and abnormal bone may be indistinct.

The most common presenting symptom is deafness. The other symptoms include postaural or temporal swelling, otorrhea, otalgia, and pulsatile tinnitus. The abnormal physical findings were unilateral conductive or sensorineural hearing loss,[3] stenosis of the ear canal, and cholesteatoma, in a series of 72 cases.[4] None of the patients with fibrous dysplasia limited to the temporal bone had abnormal pigmentation or endocrine disorders.

Diagnosis mainly depends on the radiographic findings. In a study of 39 patients[5] with biopsy-confirmed fibrous dysplasia of the skull and facial bones, three radiographic patterns were identified. One was pagetoid, with bone expansions and alternate areas of radiodensity and radiolucency. The second pattern was sclerotic, with bone expansion and a homogeneous radiodensity. The third type was cystlike — usually a round or oval lesion with a sclerotic border. The most common radiographic picture showed sclerosis of the temporal bone, which occurred in two thirds of cases. Thus, a temporal bone with homogeneous radiodensity and an expanded cortex is characteristic of fibrous dysplasia.

High-resolution CT provides greater detail of the extent of fibrous dysplasia of the temporal bone than does conventional radiography. It is useful in assessing the degree of external auditory canal stenosis, involvement of middle ear structures, presence of an associated cholesteatoma, and the extent of involve-

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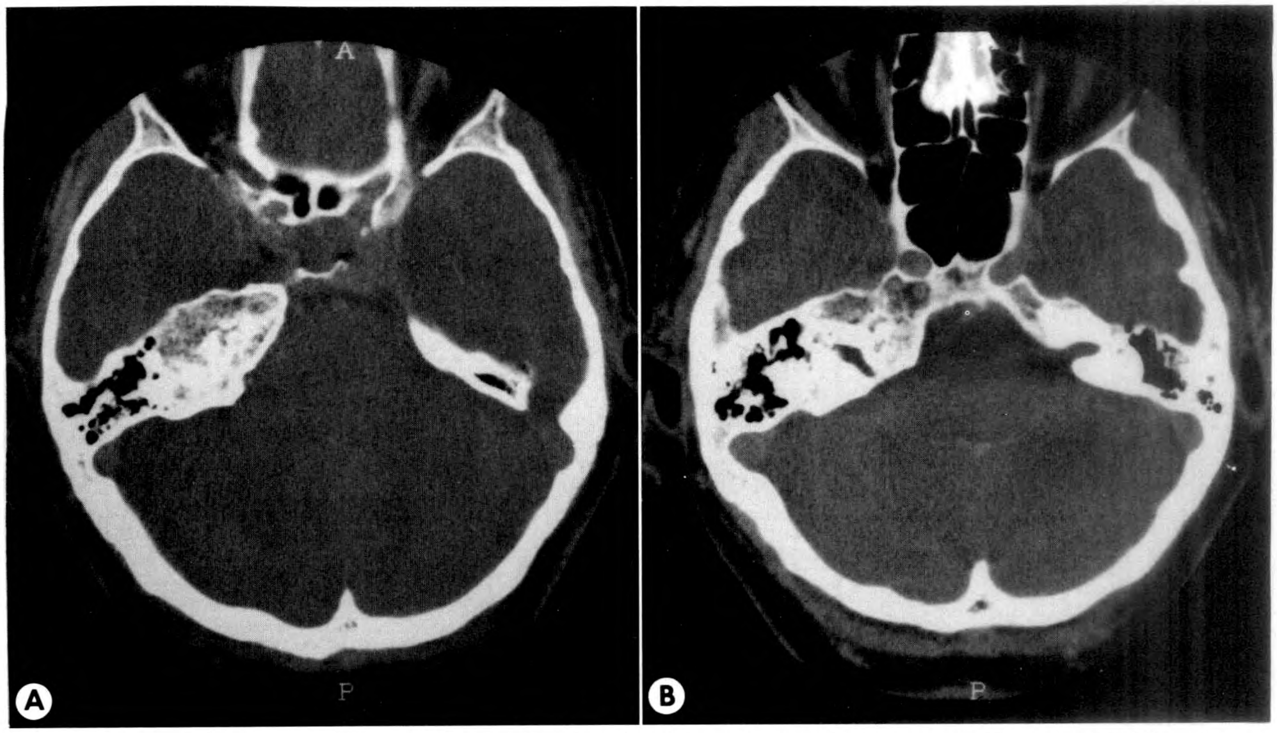

Axial computed tomograms of temporal bones demonstrating fibrous dysplasia involving right petrous temporal bone.

ment of facial nerve and inner ear structures.

Fibrous dysplasia is a benign disorder, and the lesions usually become quiescent after puberty. The complications of monostotic fibrous dysplasia of the temporal bone include chronic otitis media with or without cholesteatoma, destruction of the ossicles, labyrinthitis, sensorineural hearing loss, and facial nerve paralysis. Fibrous dysplasia can take a protracted course.[6] An 8-year-old girl who presented with a small bony swelling near the ear was followed up over a 32-year period, and fibrous dysplasia was found extensively involving the temporal, frontal, and zygomatic bones. She underwent numerous operative procedures and finally had complete peripheral facial nerve paralysis and total loss of audiovestibular function.

Malignant transformation has not been reported for temporal bone fibrous dysplasia, but it has been in other sites. In a series of 28 cases,[7] two thirds of the malignancies were osteosarcomas. The mean time interval between documentation of fibrous dysplasia and that of the sarcoma was 13.5 years. The incidence of malignant degeneration was 0.4%, and in cases with extraskeletal manifestations like cutaneous pigmentation and precocious puberty (Albright's syndrome) the incidence was 4%.

The treatment of fibrous dysplasia of any site is surgery. The main indications for fibrous dysplasia in the temporal bone are for restoration of hearing and prevention of complications from occult cholesteatoma. As fibrous dysplasia is likely to become quiescent, surgical intervention should be as conservative as possible. The patient should be informed of the unpredictability of fibrous dysplasia and the tendency for recurrence. Radiotherapy is not beneficial and can induce malignant change.

Patients with unilateral fibrous dysplasia resulting in conductive hearing loss may not need any surgical correction. Periodic CT scanning is used to assess the progress of the disease process and to detect the development of secondary disorders like cholesteatoma. In this way, surgical intervention can be more prudently advised and repeated surgery avoided.

CONCLUSIONS

Fibrous dysplasia of the temporal bone is a rare disorder; the common clinical symptoms are conductive hearing loss and postauricular swelling. The complications are usually due to secondary disorders like cholesteatoma, and rarely due to abnormal bone growth itself. Malignant degeneration of the temporal bone, although not documented, is a potential complication. Computed tomographic findings are pathognomonic and also aid in monitoring the progress of the disease. A conservative approach is recommended in patients who require surgery, and should be guided by the disability and the radiographic findings.

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TWENTY-NINTH POSTGRADUATE COURSE IN EAR SURGERY

The 29th Postgraduate Course in Ear Surgery will be held April 10-15, 1994, in Nijmegen, the Netherlands. For more information, contact Prof dr P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; fax 80-540251.

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XXIST CONGRESS OF THE INTERNATIONAL NEUROOTOLOGICAL AND EQUILIBRIOMETRIC SOCIETY

The XXIst Congress of the International Neurootological and Equilibriometric Society will be held in Bad Kissingen, Germany, March 17-20, 1994. For further information, contact NES, Prof Dr C.-F. Claussen, Kurhausstrasse 12, D-97688 Bad Kissingen, Germany; telephone (00 49) 9 71-6 48 32; fax (00 49) 9 71-6 86 37.

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PATHOLOGY CONSULTATION

DESMOPLASTIC MELANOMA

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Desmoplastic melanoma is an uncommon type of malignant melanoma that has a predilection for the skin of the head and neck. Because of a progressive metaplasia of melanocytes and an accompanying desmoplasia, the neoplasm can be mistaken for benign or malignant soft tissue lesions. The neurotropism exhibited by many desmoplastic melanomas is a strong adverse factor for prognosis; otherwise, the biologic behavior is not unlike that of conventional melanomas.

Desmoplastic melanoma continues to be a troublesome neoplasm for surgical pathologists and therapists. It most often affects the sun-exposed skin of the head and neck, but can arise in almost any cutaneous site and also in the mucosae, including the oral cavity.[1-3] In the skin of the head and neck the distribution of desmoplastic melanomas closely follows that of lentigo maligna, a lesion with which the desmoplastic melanoma is frequently associated.[2,4,5] There is a slight predilection for males, and while the peak incidence is in the seventh decade, it can present in young adults.

The desmoplastic melanoma may be associated with practically any melanocytic skin lesion, eg, atypical melanocytic proliferation, lentiginous melanoma, superficial spreading melanoma, and nodular melanoma.[4,5] In nearly half the reported cases, especially in recurrent tumors, there is no intraepidermal melanocytic component.[2,4,5] Almost 50% of the desmoplastic melanomas reported by Jain and Allen[4] had no demonstrable preexisting or coexisting melanocytic lesions and were considered "de novo" tumors.

In both its gross and microscopic appearances, primary and recurrent desmoplastic melanoma can be deceptively innocuous. The clinical presentation can be that of a discoid papule, plaque, or nodule, with or without pigment in the adjacent epidermis.[2] The size of the lesions ranges from 0.5 cm or less to over 5.0 cm. Recurrent tumors are usually more bulky and multinodular.[4-8]

The variations in histomorphology exhibited by desmoplastic melanomas are now assumed to be due to a progressive metaplasia (fibroblastic, myofibroblastic, schwann cell–like) of melanocytes, mediated by the extracellular matrix of the dermis.[2,4,5] Much of the desmoplasia and the often-seen neurotropism is considered to be a result of phenotypic derepression of the embryonic precursors of the epidermal melanocytes, the neural crest cells.[2,4-6]

The growth of a desmoplastic melanoma can simulate that of a proliferative scar. There is a vertical and lateral spread in the dermis and subcutaneous tissues (see Figure, A). Under low-magnification, light-optic examination, the tumor can look like a fibrous nodule or proliferative scar, usually with an abundant and dense collagenous tissue. In this background, however, are found elongated spindled neoplastic cells, often aligned in fascicular patterns (see Figure, B). The nuclei of these cells vary from hyperchromatic and atypical (see Figure, C) or vesicular with prominent nucleoli to a rather banal appearance. Mitoses may be seen but may be sparse. Also sparse to absent are melanin-containing cells and melanophages. Scattered, variably prominent collections of perivascular lymphoid cells are said to be useful in recognizing a desmoplastic melanoma.[2] The desmoplasia is almost always a feature and increases with recurrences.

Neurotropic features, found in many but not all desmoplastic melanomas, are characterized by either the formation of neuroidal structures by neoplastic cells or by neurotropism. The latter is a merger or blending of neoplastic spindle cells with the perineurium, or invasion of the endoneurium, or both (see Figure, D). An axonal proliferation may also be prominent.[2] A myxoid alteration in the stroma further likens the desmoplastic melanoma to a neurogenous tumor.

Bruijn et al[2] list 63 different histologic diagnoses that have been applied to desmoplastic melanomas. These misdiagnoses range from proliferative or hypertrophic scar and sclerosing blue nevus to malig-

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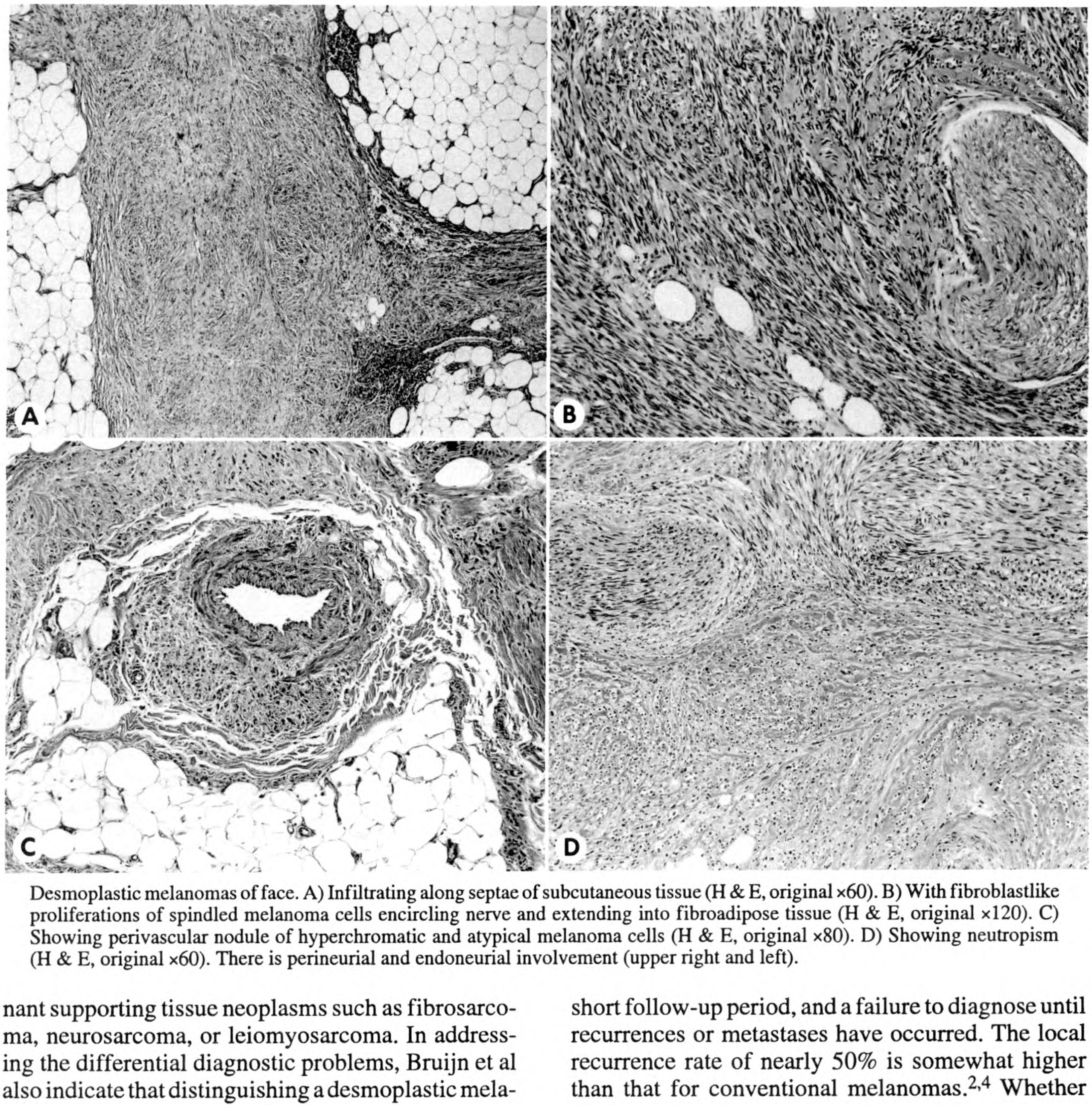
REPRINTS — John G. Batsakis, MD, Dept of Pathology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.



Desmoplastic melanomas of face. A) Infiltrating along septae of subcutaneous tissue (H & E, original ×60). B) With fibroblastlike proliferations of spindled melanoma cells encircling nerve and extending into fibroadipose tissue (H & E, original ×120). C) Showing perivascular nodule of hyperchromatic and atypical melanoma cells (H & E, original ×80). D) Showing neutropism (H & E, original ×60). There is perineurial and endoneurial involvement (upper right and left).

nant supporting tissue neoplasms such as fibrosarcoma, neurosarcoma, or leiomyosarcoma. In addressing the differential diagnostic problems, Bruijn et al also indicate that distinguishing a desmoplastic melanoma from a malignant nerve sheath tumor is most difficult, and at times may be impossible. The majority of desmoplastic melanomas will immunohistochemically color with antibodies against vimentin, S-100 protein antigen, and neuron-specific antigen. A "melanoma marker," HMB-45 is, however, frequently negative.[2] In assessing immunohistochemical data, it should be recalled that the desmoplasia is a combination of the metaplastic melanocyte and a nonneoplastic, albeit provoked, fibrosis much like that seen in sarcomatoid or metaplastic carcinomas of the upper aerodigestive tracts.

A clear perspective of the biologic course of a desmoplastic melanoma is hampered by the relatively small number of reported cases, a too often short follow-up period, and a failure to diagnose until recurrences or metastases have occurred. The local recurrence rate of nearly 50% is somewhat higher than that for conventional melanomas.[2,4] Whether this is due to advanced stage at time of diagnosis and/or incomplete surgical excision is not known. Neurotropism not only increases the local recurrence rate, it also is responsible for the morbidity and mortality of head and neck desmoplastic melanomas because of intracranial extension via the cranial nerves, particularly the fifth and seventh.[4] The histologic appearance of a metastasis is, many times, like that of a conventional melanoma, but can be desmoplastic or mixed conventional-desmoplastic.

When their tumor thickness has been measured, most desmoplastic melanomas are 4 to 6 mm and at anatomic level IV or V.[2,4] From et al[9] advise including the desmoplastic areas in assessing depth of invasion.

There have been few analyses of prognostic factors. A notable exception is the study by Smithers et al.[5] These workers found, by multivariate analyses, that local recurrences were associated with an incorrect histopathologic diagnosis, a head and neck site, less than 1-cm surgical margins of excision, a level V tumor, and a tumor thickness greater than 4 mm. Metastases were associated with the same risk factors, except for incorrect diagnosis and tumor thickness.

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SIXTH ANNUAL SYMPOSIUM ON AESTHETIC SURGERY OF THE FACE

The Sixth Annual Symposium on Aesthetic Surgery of the Face will be held in San Francisco, California, March 24-26, 1994. For registration information, please call (415) 476-5808. For program information, please call (415) 476-4251.

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CLINICAL APPLICATIONS OF VESTIBULAR SCIENCE

The conference Clinical Applications of Vestibular Science will be held in Los Angeles, California, February 12-13, 1994. For information, contact Marjorie Harris, Program Coordinator, 15-50 Rehabilitation Center, UCLA, Los Angeles, CA 90024-1651; telephone (310) 825-5131; fax (310) 206-2327.

BOOK REVIEWS

Neoplasms of the Larynx

Alfio Ferlito, editor. Hard cover, illustrated, indexed, 618 pages, 1993. Churchill Livingstone, Edinburgh, Scotland, $195.

The book *Neoplasms of the Larynx*, edited by an Italian laryngologist and friend of American laryngology, is a textbook with which most of the multinational cast of contributors can be proud to be associated.

The book is huge (618 pages). It covers the entire subject of laryngeal cancer, beginning with a comprehensive (23 pages, 13 photographs, and 152 references) historical perspective by Loring Pratt. The book ends with a chapter on psychological aspects of the disease by Frank Lucente. Between is everything, and I mean everything, ever written, thought about, researched, lectured, and practiced on the subject of larynx cancer.

The text is an interesting blend of pathology, clinical, and research subjects. The book is massively illustrated. For example, there are 46 illustrations of atypical forms of squamous cell carcinoma, 34 illustrations of salivary gland tumors of the larynx, and 47 illustrations of soft tissue neoplasms. All of the illustrations are of high quality, are black-and-white, and are printed on first-class paper.

Each subject is supported by a comprehensive bibliography. For example: the subject of cartilaginous tumors is supported by 72 references, hemopoietic neoplasms by 91, documentation by 72, imaging by 21, and Ferlito's pet subject — classification — by 54 references.

There is everything needed for reference by the pathologist. There is plenty of substance for the laryngologist: preoperative planning by Golden and Lavender, surgery by Silver, the neck by M. Friedman, laser by Shapshay, and radiation by Mendenhall and the Florida group. Each clinical subject has the same liberal use of illustrations and charts, and all have a comprehensive bibliography.

A problem with any text with multiple authors is internal contradictions between chapters. This problem is exaggerated when the authors are not only from several disciplines but also from multiple countries. For example: M. Friedman's chapter on neck metastasis is accurate in stating that the treatment of the clinical N0 neck is unsettled. Nevertheless, the radiation chapter assumes, without any supporting data, that the problem is settled "because of the ease of bilateral neck irradiation." Stell's chapter on retreatment begins by stating that "up to 50% of treatment by radiation fails and the failure rate increases with the stage." The chapter documents the problems of diagnosis of recurrence after radiation and the unsatisfactory results of surgical salvage in all stages. The Mendenhall chapter, on the other hand, has numbers that represent people jumping around like a pride of cats as an argument for the concept of "radiate and salvage," with little recognition of the problems associated with surgical salvage; the surgeons can just pick up the pieces with no problems. There is no way around this kind of contradiction except to read each chapter with the others in mind. It would be best to read the Stell retreatment chapter first.

This is a great book. I will keep it in my personal library for reference and use the wonderful photos for patient education. It belongs in all otolaryngology training program libraries. Surgical pathologists who work with a laryngologist will keep this book right next to their salivary gland pathology fascicles as a reference. (The pathologist will know why.) Alfio Ferlito has given us a masterpiece.

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Asthma and Allergy in Pregnancy and Early Infancy

Michael Schatz and Robert S. Zeiger, editors. *Allergic Disease and Therapy Series #7.* David G. Tinkelman, series editor. Hard cover, illustrated, indexed, 656 pages, 1993. Marcel Dekker, New York, NY, $165.

The joys of pregnancy and parenthood have been exalted many times, but to the allergic expectant mother and to the parents of an allergic infant, they can be frightening, self-incriminating experiences. Although not all questions are answered in this book, it does convey the most up-to-date information in an orderly and understandable fashion. The editors have compiled the knowledge of 35 colleagues in the field of allergy who represent a variety of disciplines, including those in the basic sciences and clinical research, from institutions as far away as Israel, Sweden, and Australia and from a broad expanse of North America. The result is this stocky book that presents in its 23 chapters solid background information and the most current developments. It is a most timely presentation, since immunologic findings are rapidly occurring in the expanding field of allergy.

The format of the text is straightforward and logical. The first 4 chapters cover the germane physiology of normal pregnancy, such as hormonal changes and their consequences, fetal oxygenation and acid-base balance compromised by anaphylaxis or asthma, pulmonary physiology, and immunologic changes. The next 5 chapters deal with general therapeutic principles, including those of immunotherapy, indications for psychologic care and the appropriate therapies, considerations in administering medications during pregnancy and lactation, the reproductive effects of specific medications for asthma and allergies, and the medicolegal considerations of prescribing medications to be used during pregnancy. Chapter 11 in this group of chapters is unique in a medical text; it is devoted entirely to a critical review of the literature related to the book's topic, specifically the effects of pregnancy on asthma. The authors nicely summarize their findings in helpful charts and graphs. In chapters 14 through 17, the effects of varied other allergies and immunologically mediated adverse drug reactions in pregnancy are described.

Asthma and allergy in early infancy is not addressed until the last 6 chapters of this text, during which a review of genetic and environmental factors and the prevention of allergy in children is given, and the natural history of allergic disease is discussed. Food hypersensitivity and wheezing in infancy were each considered worthy of their own chapter.

The closing pages are an appendix offering the 1992 guidelines set forth by the National Asthma Education Program Working Group on Asthma and Pregnancy for the management of gestational asthma. The text is seldom interrupted by illustrations, most of which are graphs or charts. Each chapter is finalized by an adequate, if not extensive, bibliography, which increases the value of the book as a source of reference. While not essential to their practice, this would be a useful reference book to otolaryngologists, since most of them see pregnant patients and young infants from time to time.

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Color Atlas of Anatomy

Johannes W. Rohen and Chichiro Yokochi. Third edition. Hard cover, illustrated, indexed, 484 pages, 1993. Igaku-Shoin Medical Publishers, New York, NY.

Dissection Manual: Companion to Rohen/Yokochi Color Atlas of Anatomy

Jack L. Wilson. Soft cover, illustrated, 273 pages, 1993. Igaku-Shoin Medical Publishers, New York, NY.

The first edition of this atlas was published in 1983. At that time the authors' justification for adding to the existing supply of anatomic atlases was threefold: 1) to present photographic illustrations that reflect the third dimension and, therefore, are more realistic than the schematic drawings that constituted most of the atlases up to that time, 2) to portray macroscopic anatomy as it relates to the regional and stratigraphic aspects of the object itself, and 3) to augment the sparse supply of cadavers (compared to the increased number of medical students) by making available the next-best thing: realistic macroscopic photographs.

The authors' intent and goals did not change during the publication of the second edition, in 1987, nor has it for this 1993 edition. Computed tomography (CT) scan figures and magnetic resonance (MR) images were added to the second edition, and this third edition includes a number of additional new macrophotographs, CT scans, and MR images, along with many new schematic drawings. For each region of the body, a topographic chapter is preceded by its corresponding systematic chapter; this format enhances the book's application to any course of study.

Each photograph, with its three-dimensional impression, exhibits artistic skill, and those of human specimens are aesthetic highlights. The section on anatomic dissection of the head and neck is generally extensive and excellent, with particularly vivid details. The photographs of ear anatomy, although consisting of splendid displays of minute structures, were taken at too great a distance to make them explicit, however. Even an expert anatomist will need to check the caption to be sure of the identity of many of the structures in these.

The now-frequent use of CT scans and MR imaging has increased the need to know human anatomy, and this remarkable and beautifully done compendium is particularly recommended for today's clinical work.

The dissection manual that accompanies the atlas provides supplemental basic line dissection instructions, and points out related features to be observed during dissection. The text is divided into regional chapters and gives in parentheses the corresponding page numbers in the atlas. Although it has the same limitations of handbooks prepared for similar use, specifically the disadvantage of referring from one book to the other, this manual compares favorably with similar texts.

Dennis G. Pappas, MD
Birmingham, Alabama

Tonsils: A Clinically Oriented Update

G. B. Galioto, editor. E. Mevio, P. Galioto, M. Benazzo, coeditors. Hard cover, illustrated, indexed, 349 pages, 1992. Karger, Basel, Switzerland.

Controversial issues clinicians would like answered on this topic include the following. What is the indication for adenoidectomy in allergic children? Is the adenoid tissue, by virtue of hypertrophy and/or infection, the source of chronicity of otitis media? What is the indication for tonsillectomy and adenoidectomy in patients with obstructive sleep apnea? Or in heavy-snoring children? If the tonsils have such a capacity for immunologic function, why is it obvious that with chronic inflammation they play an important role in the occurrence of secondary disease in the form of focal infection? Last, how far are we from a vaccine panel to treat diseases such as tonsillitis and severe otitis media?

Although this book provides no answers to these questions, it does convey up-to-date information and insights that will familiarize the specialist with the broad spectrum of disease found in this anatomic region.

The concise essays in this small volume are the result of presentations from the second International Symposium on Tonsils held in Pavia in September 1991. The editor has divided 60 papers into six sections. The aim of the first section is to identify those morphological and developmental elements associated with immunologic-bearing lymphocytes. The development, microvasculature, and comparative anatomy are presented.

In recent years, a more profound knowledge of the tonsils and adenoids has emerged as a result of immunohistochemical studies. These new techniques and their immune activities are discussed in the second section. Tonsillar infection, focal infection of tonsil and adenoid, and tonsillar hypertrophy and its complications are three sections that capture the clinically updated research on this subject. Finally, medical and surgical therapy is highlighted as in the effects of tonsillectomy, a modern approach to therapy of streptococcus pharyngotonsillitis, clinical trial results for surgery for otitis media, adenotonsillectomy for facial growth dysfunction, and others.

I agree with the title that this is a particularly clinically oriented text. From studies of recent years one can accept accordingly the favorable or unfavorable effects of adenoidectomy, eg, decreases or no change on recurrence rate of middle ear effusion, improvements in hearing, and decreases or no change in negative middle ear pressure. There is an interesting discussion in this book that finds that the cure rate fiom otitis media with effusion became quite similar between the adenoidectomized and nonadenoidectomized groups by the third year. That it was better in the adenoidectomized group for the first 2 years means that the surgery shortens the time for a cure to under 3 years.

The typical format of Introduction, Methods, Material, Results, and Discussion is used in a short paragraph fashion. Much of the text is adequately illustrated with diagrams and microscopic illustrations. This is a book that should well serve the busy practitioner, especially the otolaryngologist and pediatrician, the immunologist, and the resident first encountering these diseases.

Dennis G. Pappas, MD
Birmingham, Alabama

Neuromonitoring in Otology and Head and Neck Surgery

Jack M. Kartush and Kenneth R. Bouchard, editors. Hard cover, illustrated, indexed, 268 pages, 1991. Raven Press, New York, NY, $80.

The facial nerve, hearing, vision, and vocal cords are all subjected to the risk of neural injury during otologic and head and neck surgery. Preventative monitoring is employed in operations for acoustic neuroma removal (to conserve hearing and observe function of the facial nerve, which may be inconspicuously stretched), following temporal bone trauma (where there may be distortion of tissue), for correction of external auditory atresia (where the facial nerve follows an unusual course), and for extensive cholesteatoma (in which the facial canal may be eroded or covered with granulation tissue). Experiences such as these in monitoring the facial nerve led to the monitoring of lower cranial nerves in thyroidectomy and neck dissections. As yet, there is no visual-evoked response for sinus surgery.

The editors are to be commended for their straightforward, no-nonsense presentation of neuromonitoring as an adjunct, not a replacement, for experienced surgery of the facial nerve. They do not advocate this evolving science as a standard of care, nor do they recommend specific monitoring devices for certain operations (eg, electromyography for stretching of the facial nerve in posterior fossa versus a mechanical pressure device as in chronic ear disease). Their recommendations, however, would have added clinical information for the reader, especially if they had included the costs.

False-negative outcomes are reviewed, and give insight into appropriate monitoring techniques and motion detectors. Claims are made that false-negative outcomes are few, but probably most are not even reported in the literature.

This book is limited in size by its special subject matter — the principles of neurophysiologic monitoring and their current applications. The 12 chapters could be divided into a basic science section (3 chapters) and a clinical application section (9 chapters). All aspects of neuromuscular testing are discussed, including the goals of intraoperative facial nerve testing, artifacts of a biologic and nonbiologic nature, various needle electrode configurations, and the like. It is indeed a pleasure to read Aage R. Møller's excellent description of the physiology of hearing, which he presents in a simple, understandable fashion.

The editors have compiled a superb collection of diverse information in what may well be the authoritative text on neuromonitoring — certainly one that will serve as a reference work. Although many of the authors have previously published on their subject matter, most of the material in this book appears to be not previously published. All those who perform otologic and/or head and neck surgery should own or at least read this book.

Dennis G. Pappas, MD
Birmingham, Alabama

Surgical Techniques of the Temporal Bone and Skull Base

Herbert Silverstein and Seth Rosenberg. Hard cover, illustrated, indexed, 240 pages, 1992. Lea & Febiger, Philadelphia, Pa.

This book reflects the philosophy, approach, and techniques of the senior author regarding treatment of various ear diseases. Furthermore, it is more or less a compendium of the developments and teachings of Dr Silverstein, who has been a prolific contributor to the otologic-neurologic literature in both the clinical-surgical and basic science areas, and a productive innovator in developing and improving instruments and appliances.

Each of the 19 chapters is informative and concisely written without the distraction of minutiae. They would fit nicely into three segments: introduction (medical-legal aspects, operating room setup, and intraoperative monitoring), otology, and neuro-otology. The authors adhere to the theme of their title in limiting their coverage to aspects of surgical procedures involving the temporal bone and skull base.

There is an excellent chapter on facial monitoring. It conveys useful criteria for nerve stimulation and justification for the appropriateness of various stimulators in otologic or posterior fossa facial nerve surgery. The authors claim that the monitor-stimulator has helped prevent injury to the exposed facial nerve in 20 cases, and that there has been no case of immediate postoperative facial weakness in 500 cases of chronic ear disease. I daresay that otologic surgeons in general can make the same claim without the use of that equipment.

The authors also present the reasoning behind their methods, which is another of this book's attributes. One of the innovative techniques they endorse is an office procedure using intravenous fluorescein to diagnose a fistula. A glass micropipet is introduced near the oval window through a cruciate myringotomy incision. However, it is noted by the authors that as with other fistula diagnostic procedures, many pitfalls exist. Incidentally, "pitfalls," "axioms," and "algorithms" are sometimes strategically placed at the end of the chapter, which is a very skillful way to add a great deal of information in very few words.

I might quibble with the authors about performing an atticotomy with a canal wall intact mastoidectomy (removal of the head of the malleus and the incus). Does a retraction cholesteatoma not develop? At any rate, each surgeon has developed his or her own technique for such surgery, and the authors assure us that theirs has proven to give good results. (Cartilage is placed in the attic to prevent this retraction.)

The print used in this neatly done atlas-type volume is easily read, and there are only occasional typographic errors. Certainly, the young practicing otologist or those still in training or studying this field will find it useful. The more seasoned otoneurologist-neurosurgeon team will benefit from some specifics in otoneurologic approaches. There is a good description of the anatomy of the venous system of the skull base. A videotape has been produced to be used in conjunction with this book, but was not available for review. Its intent is to enhance the reader's understanding of the techniques described in the text.

Dennis G. Pappas, MD
Birmingham, Alabama

INSTRUCTIONS TO AUTHORS

SUBMISSION

Send manuscripts to Editor Brian F. McCabe, MD, Department of Otolaryngology–Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242. Original manuscripts dealing with clinical and scientific aspects of otolaryngology, bronchoesophagology, head and neck medicine and surgery, maxillofacial and plastic surgery, audiology, speech pathology, and related specialties are considered for publication. All materials submitted for publication undergo peer review.

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Note: For information on otitis media caused by *Streptococcus pneumoniae*, see **CLINICAL STUDIES** section.
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In clinical trials of otitis media in nearly 400 children between the ages of 6 months to 10 years, *S pneumoniae* was isolated from 47% of the patients, *H influenzae* from 34%, *M (B) catarrhalis* from 15%, and *S pyogenes* from 4%.

The overall response rate of *S pneumoniae* to cefixime was approximately 10% lower and that of *H influenzae* or *M (B) catarrhalis* approximately 7% higher (12% when beta-lactamase positive strains of *H influenzae* are included) than the response rates of these organisms to the active control drugs.

In these studies, patients were randomized and treated with either cefixime at dose regimens of 4 mg/kg BID or 8 mg/kg QD, or with a standard antibiotic regimen. Sixty-nine percent to 70% of the patients in each group had resolution of signs and symptoms of otitis media when evaluated 2 to 4 weeks posttreatment, but persistent effusion was found in 15% of the patients. When evaluated at the completion of therapy, 17% of patients receiving cefixime and 14% of patients receiving effective comparative drugs (18% including those patients who had *H influenzae* resistant to the control drug and who received the control antibiotic) were considered to be treatment failures. By the 2- to 4-week follow-up, a total of 30% to 31% of patients had evidence of either treatment failure or recurrent disease.

Bacteriological Outcome of Otitis Media at 2 to 4 Weeks Posttherapy
Based on Repeat Middle Ear Fluid Culture or
Extrapolation from Clinical Outcome

Organism	Cefixime[a] 4 mg/kg BID	Cefixime[a] 8 mg/kg QD	Control[a] drugs
Streptococcus pneumoniae	48/70 (69%)	18/22 (82%)	82/100 (82%)
Haemophilus influenzae beta-lactamase negative	24/34 (71%)	13/17 (76%)	23/34 (68%)
Haemophilus influenzae beta-lactamase positive	17/22 (77%)	9/12 (75%)	1/1[b]
Moraxella (Branhamella) catarrhalis	26/31 (84%)	5/5	18/24 (75%)
S pyogenes	5/5	3/3	6/7
All Isolates	120/162 (74%)	48/59 (81%)	130/166 (78%)

[a] Number eradicated/number isolated.
[b] An additional 20 beta-lactamase positive strains of *H influenzae* were isolated, but were excluded from this analysis because they were resistant to the control antibiotic. In 19 of these, the clinical course could be assessed, and a favorable outcome occurred in 10. When these cases are included in the overall bacteriological evaluation of therapy with the control drugs, 140/185 (76%) of pathogens were considered to be eradicated.

Tablets should not be substituted for suspension when treating otitis media.

CONTRAINDICATIONS

SUPRAX is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH SUPRAX IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO SUPRAX OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES, AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Administer cautiously to allergic patients.

Treatment with broad-spectrum antibiotics, including SUPRAX, alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of severe antibiotic-associated diarrhea including pseudomembranous colitis.

Pseudomembranous colitis has been reported with the use of SUPRAX and other broad-spectrum antibiotics (including macrolides, semisynthetic penicillins, and cephalosporins); therefore, it is important to consider this diagnosis in patients who develop diarrhea in association with the use of antibiotics. Symptoms of pseudomembranous colitis may occur during or after antibiotic treatment and may range in severity from mild to life-threatening. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, management should include fluids, electrolytes, and protein supplementation. If the colitis does not improve after the drug has been discontinued, or if the symptoms are severe, oral vancomycin is the drug of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes of colitis should be excluded.

PRECAUTIONS

General: Use, especially when prolonged, may result in overgrowth of resistant organisms. If superinfection occurs during therapy, take appropriate measures.

Carefully monitor patients on dialysis. Adjust dosage of SUPRAX in patients with renal impairment and those undergoing continuous ambulatory peritoneal dialysis and hemodialysis. (See **DOSAGE AND ADMINISTRATION** in package insert.)

Prescribe cautiously in patients with a history of gastrointestinal disease, particularly colitis.
Drug Interactions: No significant drug interactions have been reported to date.
Drug/Laboratory Test Interactions: A false-positive reaction for ketones in the urine may occur with tests using nitroprusside but not with those using nitroferricyanide.

SUPRAX administration may result in a false-positive reaction for glucose in the urine using Clinitest®,** Benedict's solution, or Fehling's solution. Use glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix®** or Tes-Tape®**).

A false-positive direct Coombs test has been reported during treatment with other cephalosporin antibiotics; therefore, it should be recognized that a positive Coombs test may be due to the drug.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Although no lifetime animal studies have been conducted to evaluate carcinogenic potential, no mutagenic potential of SUPRAX was found in standard laboratory tests. In rats, reproductive studies revealed no fertility impairment at doses up to 125 times the adult therapeutic dose.
Usage in Pregnancy: *Pregnancy Category B:* Reproduction studies have been performed in mice and rats at doses up to 400 times the human dose and have revealed no evidence of harm to the fetus due to SUPRAX. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
Labor and Delivery: SUPRAX has not been studied for use during labor and delivery. Treatment should only be given if clearly needed.
Nursing Mothers: It is not known whether SUPRAX is excreted in human milk. Consider discontinuing nursing temporarily during treatment with this drug.
Pediatric Use: Safety and effectiveness of SUPRAX in children aged less than 6 months have not been established.

The incidence of gastrointestinal adverse reactions, including diarrhea and loose stools, in pediatric patients receiving the suspension was comparable to that seen in adult patients receiving tablets.

ADVERSE REACTIONS

Most adverse reactions observed in clinical trials were of a mild and transient nature. Five percent (5%) of patients in the US trials discontinued therapy because of drug-related adverse reactions. The most commonly seen adverse reactions in US trials of the tablet formulation were gastrointestinal events, which were reported in 30% of adult patients on either the BID or the QD regimen. Clinically mild gastrointestinal side effects occurred in 20% of all patients, moderate events occurred in 9% of all patients, and severe adverse reactions occurred in 2% of all patients. Individual event rates included diarrhea 16%, loose or frequent stools 6%, abdominal pain 3%, nausea 7%, dyspepsia 3%, and flatulence 4%. The incidence of gastrointestinal adverse reactions, including diarrhea and loose stools, in pediatric patients receiving the suspension was comparable to that seen in adult patients receiving tablets.

These symptoms usually responded to symptomatic therapy or ceased when SUPRAX was discontinued.

Several patients developed severe diarrhea and/or documented pseudomembranous colitis, and a few required hospitalization.

The following adverse reactions have been reported following the use of SUPRAX. Incidence rates were less than 1 in 50 (less than 2%), except as noted above for gastrointestinal events.
Gastrointestinal: Diarrhea, loose stools, abdominal pain, dyspepsia, nausea, and vomiting. Several cases of documented pseudomembranous colitis were identified during the studies. The onset of pseudomembranous colitis symptoms may occur during or after therapy.
Hypersensitivity Reactions: Skin rashes, urticaria, drug fever, and pruritus. Erythema multiforme, Stevens-Johnson syndrome, and serum sickness-like reactions have been reported.
Hepatic: Transient elevations in SGPT, SGOT, and alkaline phosphatase.
Renal: Transient elevations in BUN or creatinine.
Central Nervous System: Headaches or dizziness.
Hemic and Lymphatic Systems: Transient thrombocytopenia, leukopenia, and eosinophilia. Prolongation in prothrombin time was seen rarely.
Other: Genital pruritus, vaginitis, candidiasis.

The following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics:

Adverse Reactions: Allergic reactions including anaphylaxis, toxic epidermal necrolysis, superinfection, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemolytic anemia, hemorrhage, and colitis.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see **DOSAGE AND ADMINISTRATION** and **OVERDOSAGE**). If seizures associated with drug therapy occur, discontinue drug. Administer anticonvulsant therapy if clinically indicated.

Abnormal Laboratory Tests: Positive direct Coombs test, elevated bilirubin, elevated LDH, pancytopenia, neutropenia, agranulocytosis.

OVERDOSAGE

Gastric lavage may be indicated; otherwise, no specific antidote exists. Cefixime is not removed in significant quantities from the circulation by hemodialysis or peritoneal dialysis. Adverse reactions in small numbers of healthy adult volunteers receiving single doses up to 2 g of SUPRAX did not differ from the profile seen in patients treated at the recommended doses.

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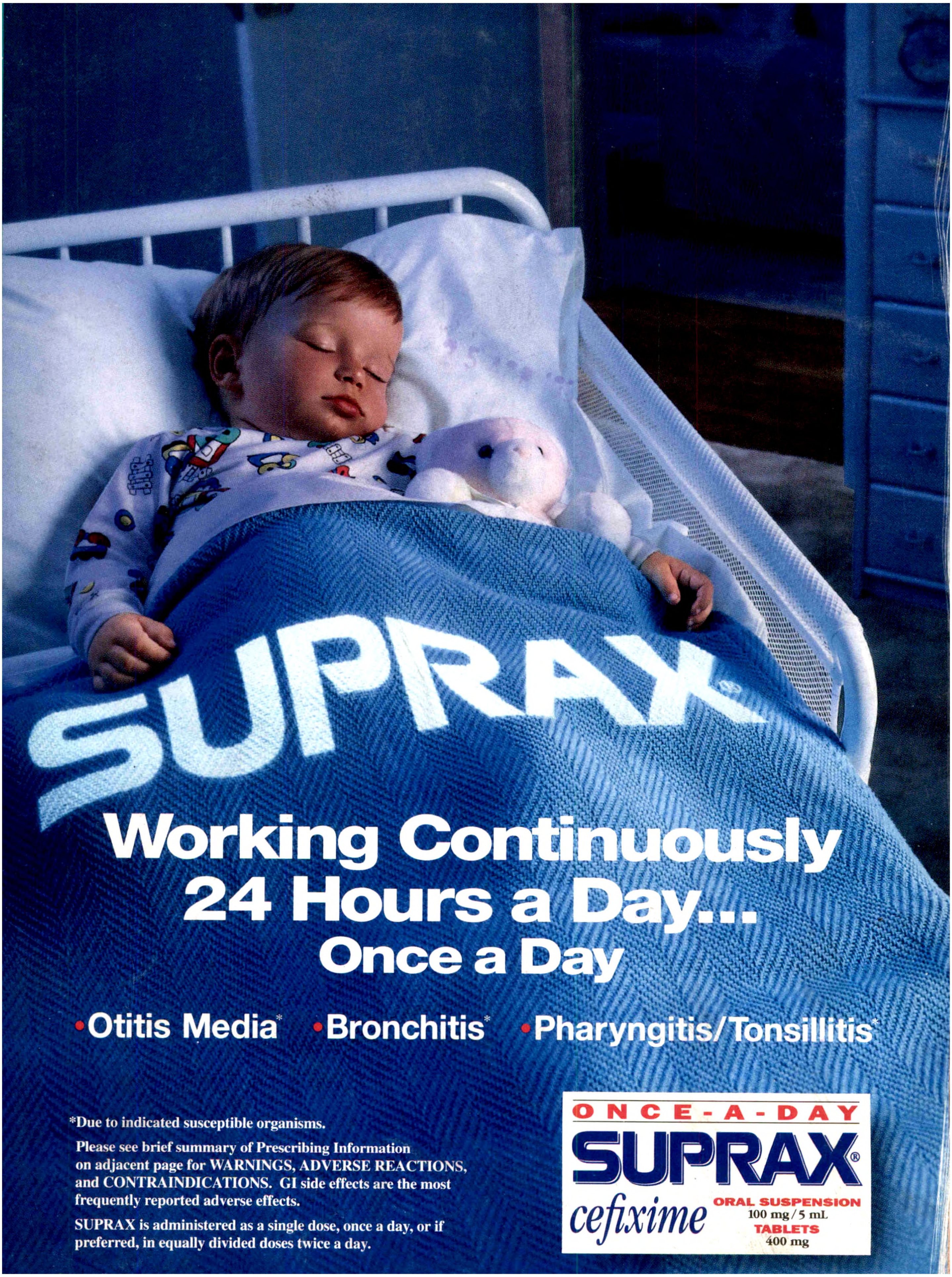

*Due to indicated susceptible organisms.

Please see brief summary of Prescribing Information on adjacent page for WARNINGS, ADVERSE REACTIONS, and CONTRAINDICATIONS. GI side effects are the most frequently reported adverse effects.

SUPRAX is administered as a single dose, once a day, or if preferred, in equally divided doses twice a day.

UCM

VOLUME 103 (2) — FEBRUARY 1994

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* The incidence of sedation with CLARITIN Tablets (8%) was similar to that of placebo (6%) at the recommended dose.

In studies with CLARITIN Tablets at doses 2 to 4 times higher than the recommended dose of 10 mg, a dose-related increase in the incidence of somnolence was observed.

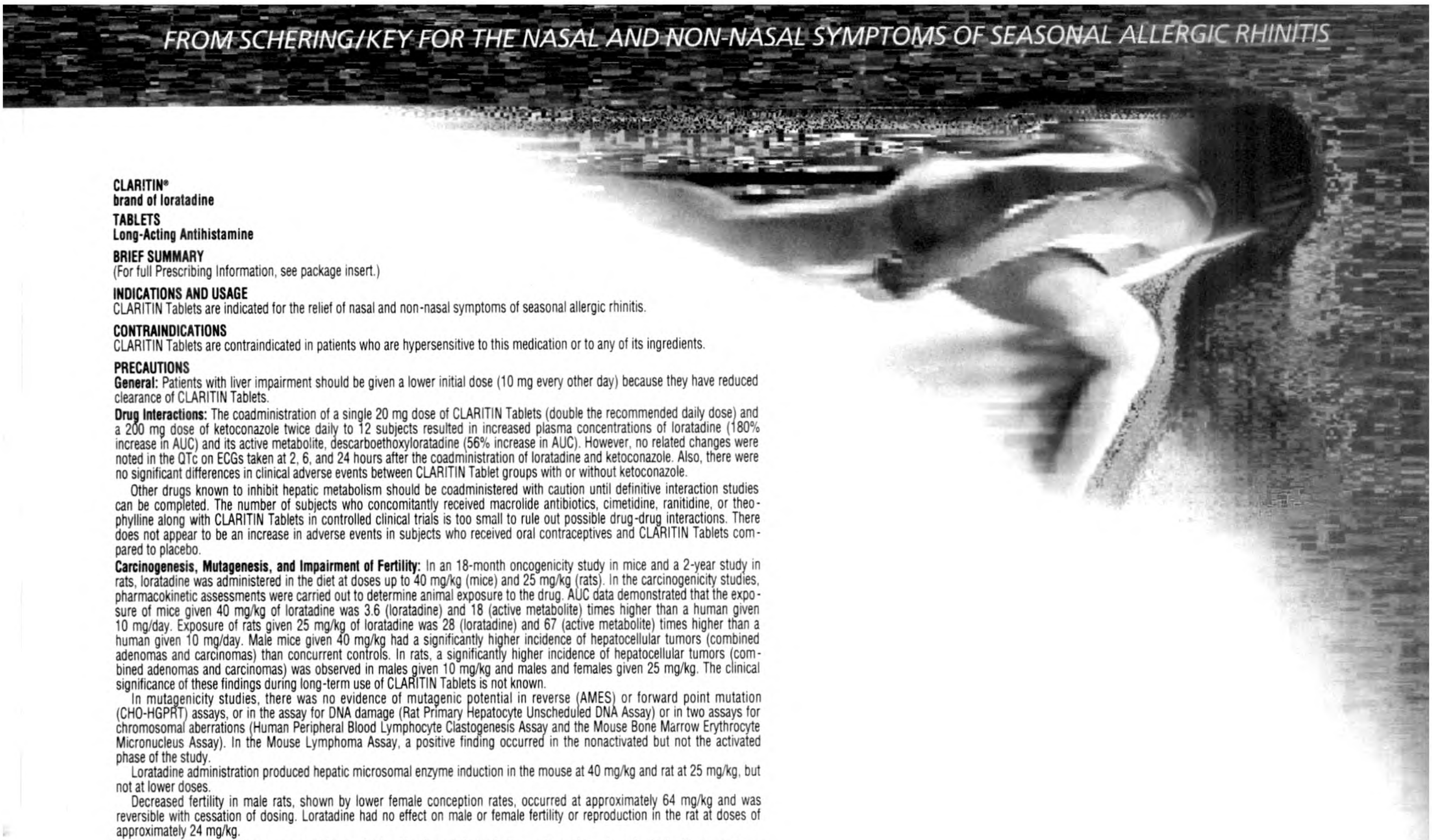
† Relief began in 13% of treated patients vs 4% of placebo-treated patients within 30 minutes (P=.04). At 2 hours, 65% of treated patients vs 48% of patients receiving placebo experienced relief. Distribution of onset times was significantly earlier for CLARITIN Tablets vs placebo (P=.03).

Once-a-day

Claritin®

10 mg TABLETS (loratadine)

Please see following page for full Prescribing Information.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brand of loratadine

TABLETS
Long-Acting Antihistamine

BRIEF SUMMARY

(For full Prescribing Information, see package insert.)

INDICATIONS AND USAGE

CLARITIN Tablets are indicated for the relief of nasal and non-nasal symptoms of seasonal allergic rhinitis.

CONTRAINDICATIONS

CLARITIN Tablets are contraindicated in patients who are hypersensitive to this medication or to any of its ingredients.

PRECAUTIONS

General: Patients with liver impairment should be given a lower initial dose (10 mg every other day) because they have reduced clearance of CLARITIN Tablets.

Drug Interactions: The coadministration of a single 20 mg dose of CLARITIN Tablets (double the recommended daily dose) and a 200 mg dose of ketoconazole twice daily to 12 subjects resulted in increased plasma concentrations of loratadine (180% increase in AUC) and its active metabolite, descarboethoxyloratadine (56% increase in AUC). However, no related changes were noted in the QTc on ECGs taken at 2, 6, and 24 hours after the coadministration of loratadine and ketoconazole. Also, there were no significant differences in clinical adverse events between CLARITIN Tablet groups with or without ketoconazole.

Other drugs known to inhibit hepatic metabolism should be coadministered with caution until definitive interaction studies can be completed. The number of subjects who concomitantly received macrolide antibiotics, cimetidine, ranitidine, or theophylline along with CLARITIN Tablets in controlled clinical trials is too small to rule out possible drug-drug interactions. There does not appear to be an increase in adverse events in subjects who received oral contraceptives and CLARITIN Tablets compared to placebo.

Carcinogenesis, Mutagenesis, and Impairment of Fertility: In an 18-month oncogenicity study in mice and a 2-year study in rats, loratadine was administered in the diet at doses up to 40 mg/kg (mice) and 25 mg/kg (rats). In the carcinogenicity studies, pharmacokinetic assessments were carried out to determine animal exposure to the drug. AUC data demonstrated that the exposure of mice given 40 mg/kg of loratadine was 3.6 (loratadine) and 18 (active metabolite) times higher than a human given 10 mg/day. Exposure of rats given 25 mg/kg of loratadine was 28 (loratadine) and 67 (active metabolite) times higher than a human given 10 mg/day. Male mice given 40 mg/kg had a significantly higher incidence of hepatocellular tumors (combined adenomas and carcinomas) than concurrent controls. In rats, a significantly higher incidence of hepatocellular tumors (combined adenomas and carcinomas) was observed in males given 10 mg/kg and males and females given 25 mg/kg. The clinical significance of these findings during long-term use of CLARITIN Tablets is not known.

In mutagenicity studies, there was no evidence of mutagenic potential in reverse (AMES) or forward point mutation (CHO-HGPRT) assays, or in the assay for DNA damage (Rat Primary Hepatocyte Unscheduled DNA Assay) or in two assays for chromosomal aberrations (Human Peripheral Blood Lymphocyte Clastogenesis Assay and the Mouse Bone Marrow Erythrocyte Micronucleus Assay). In the Mouse Lymphoma Assay, a positive finding occurred in the nonactivated but not the activated phase of the study.

Loratadine administration produced hepatic microsomal enzyme induction in the mouse at 40 mg/kg and rat at 25 mg/kg, but not at lower doses.

Decreased fertility in male rats, shown by lower female conception rates, occurred at approximately 64 mg/kg and was reversible with cessation of dosing. Loratadine had no effect on male or female fertility or reproduction in the rat at doses of approximately 24 mg/kg.

Pregnancy Category B: There was no evidence of animal teratogenicity in studies performed in rats and rabbits. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, CLARITIN Tablets should be used during pregnancy only if clearly needed.

Nursing Mothers: Loratadine and its metabolite, descarboethoxyloratadine, pass easily into breast milk and achieve concentrations that are equivalent to plasma levels with an AUC_{milk}/AUC_{plasma} ratio of 1.17 and 0.85 for the parent and active metabolite, respectively. Following a single oral dose of 40 mg, a small amount of loratadine and metabolite was excreted into the breast milk (approximately 0.03% of 40 mg over 48 hours). A decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother. Caution should be exercised when CLARITIN Tablets are administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children below the age of 12 years have not been established.

ADVERSE REACTIONS

Approximately 90,000 patients received CLARITIN Tablets 10 mg once daily in controlled and uncontrolled studies. Placebo-controlled clinical trials at the recommended dose of 10 mg once a day varied from 2 weeks' to 6 months' duration. The rate of premature withdrawal from these trials was approximately 2% in both the treated and placebo groups.

REPORTED ADVERSE EVENTS WITH AN INCIDENCE OF MORE THAN 2% IN
PLACEBO-CONTROLLED ALLERGIC RHINITIS CLINICAL TRIALS
PERCENT OF PATIENTS REPORTING

	LORATADINE 10 mg QD n = 1926	**PLACEBO** n = 2545	**CLEMASTINE** 1 mg BID n = 536	**TERFENADINE** 60 mg BID n = 684
Headache	12	11	8	8
Somnolence	8	6	22	9
Fatigue	4	3	10	2
Dry Mouth	3	2	4	3

Adverse event rates did not appear to differ significantly based on age, sex, or race, although the number of non-white subjects was relatively small.

In addition to those adverse events reported above, the following adverse events have been reported in 2% or fewer patients.

Autonomic Nervous System Altered salivation, increased sweating, altered lacrimation, hypoesthesia, impotence, thirst, flushing.
Body As A Whole Conjunctivitis, blurred vision, earache, eye pain, tinnitus, asthenia, weight gain, back pain, leg cramps, malaise, chest pain, rigors, fever, aggravated allergy, upper respiratory infection, angioneurotic edema.
Cardiovascular System Hypotension, hypertension, palpitations, syncope, tachycardia.
Central and Peripheral Nervous System Hyperkinesia, blepharospasm, paresthesia, dizziness, migraine, tremor, vertigo, dysphonia.
Gastrointestinal System Abdominal distress, nausea, vomiting, flatulence, gastritis, constipation, diarrhea, altered taste, increased appetite, anorexia, dyspepsia, stomatitis, toothache.
Musculoskeletal System Arthralgia, myalgia.
Psychiatric Anxiety, depression, agitation, insomnia, paroniria, amnesia, impaired concentration, confusion, decreased libido, nervousness.
Reproductive System Breast pain, menorrhagia, dysmenorrhea, vaginitis.
Respiratory System Nasal dryness, epistaxis, pharyngitis, dyspnea, nasal congestion, coughing, rhinitis, hemoptysis, sinusitis, sneezing, bronchospasm, bronchitis, laryngitis.
Skin and Appendages Dermatitis, dry hair, dry skin, urticaria, rash, pruritus, photosensitivity reaction, purpura.
Urinary System Urinary discoloration, altered micturition.

In addition, the following spontaneous adverse events have been reported rarely during the marketing of loratadine: peripheral edema; abnormal hepatic function, including jaundice, hepatitis, and hepatic necrosis; alopecia; seizures; breast enlargement; erythema multiforme; and anaphylaxis.

OVERDOSAGE

Somnolence, tachycardia, and headache have been reported with overdoses greater than 10 mg (40 to 180 mg). In the event of overdosage, general symptomatic and supportive measures should be instituted promptly and maintained for as long as necessary.

Treatment of overdosage would reasonably consist of emesis (ipecac syrup), except in patients with impaired consciousness, followed by the administration of activated charcoal to absorb any remaining drug. If vomiting is unsuccessful, or contraindicated, gastric lavage should be performed with normal saline. Saline cathartics may also be of value for rapid dilution of bowel contents. Loratadine is not eliminated by hemodialysis. It is not known if loratadine is eliminated by peritoneal dialysis.

Oral LD_{50} values for loratadine were greater than 5000 mg/kg in rats and mice. Doses as high as 10 times the recommended clinical doses showed no effects in rats, mice, and monkeys.

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Ann Otol Rhinol Laryngol 103:1994

NEW CLINICAL SEVERITY STAGING SYSTEM FOR CANCER OF THE LARYNX

FIVE-YEAR SURVIVAL RATES

JAY F. PICCIRILLO, MD
ST LOUIS, MISSOURI
CLARENCE T. SASAKI, MD
NEW HAVEN, CONNECTICUT
CAROLYN K. WELLS, MPH
NEW HAVEN, CONNECTICUT
ALVAN R. FEINSTEIN, MD
NEW HAVEN, CONNECTICUT

Although statistics for cancer of the larynx are reported according to the tumor, node, metastasis (TNM) morphological staging system, functional clinical distinctions can identify major prognostic differences within the same morphological stage. This study was done to improve the staging system by incorporating pertinent clinical variables. In 193 patients with cancer of the larynx first treated between 1973 and 1985, the total 5-year survival was 66% (127/193). By TNM stage it was I, 78% (60/77); II, 67% (32/48); III, 60% (27/45); and IV, 35% (8/23). In three new functional severity stages that combined symptom severity and comorbidity, the corresponding rates were alpha, 83% (89/107); beta, 58% (34/59); and gamma, 15% (4/27). The functional severity stages could be combined with TNM stages to create a powerful new clinical severity staging system, in which the survival results were A, 88% (53/60); B, 80% (24/30); C, 63% (38/60); and D, 28% (12/43). These results demonstrate that inclusion of clinical variables in a formal staging system can strikingly improve prognostic estimations and classification of patients.

KEY WORDS — comorbidity, laryngeal neoplasms, neoplasm staging, neoplasms, prognosis, squamous cell carcinoma, survival rate.

INTRODUCTION

The classification of cancer, which has traditionally emphasized the gross[1-5] and microscopic[6-9] anatomic characteristics of the tumor, has led to various combinations of primary tumor (T), node (N), and metastasis (M) categories for the TNM stages of head and neck tumors.[10]

Many studies have shown, however, that the precise descriptions of anatomy do not always correspond to the clinical behavior of a neoplasm,[11-17] and that classifying a tumor is not the same as classifying a patient.

In fact, the originator of the TNM concept, Denoix, recognized this distinction when he wrote, with Schwartz,

> ... the so-called stage of the disease at the time of diagnosis or treatment should reflect not only the extension of the neoplasm, but also the type of tumor, the relation between the tumor and the illness, the host, and the time interval elapsed between the first symptom observed by the patient and the time of the diagnosis or the treatment. The vital prognostication of the cancer cannot be determined by the classification of the extension of the tumor or even by the histologic grade of malignancy.[18(p416)]

Nevertheless, cancer patients have continued to be categorized solely by the anatomic extent of their tumor, and little formal attention has been given to the functional effects of the tumor or to its coexisting associated major ailments[19-21] (also J. F. Piccirillo and A. R. Feinstein, unpublished observations).

During the past 20 years, new systems of taxonomy have been created for classifying symptoms and comorbidities.[22-27] These taxonomies can increase the scientific precision of the clinical information,[28] particularly when combined with advances in data processing and biostatistical analyses. Innovative techniques have been used to study and to improve precision in both prognosis and evaluation of treatment for a variety of human cancers, including lung,[29] breast,[20] rectum,[30,31] prostate,[32] endometrium,[21] acute leukemia,[33] osteosarcoma,[34] and nasopharyngeal carcinoma.[17,35]

Although staging of patients with laryngeal cancer continues to be based on morphology and vocal cord function, earlier work[36] by two of the present authors (C.K.W., A.R.F.) showed that the precision of morphological staging for this cancer was improved by

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From the Robert Wood Johnson Clinical Scholars Program (Piccirillo, Wells, Feinstein) and the Departments of Surgery (Otolaryngology [Sasaki, Piccirillo]) and Medicine and Epidemiology (Feinstein), Yale University School of Medicine, New Haven, Connecticut. Dr Piccirillo is the recipient of the 1993 Junior Faculty Research Award from the American Laryngological Association and a Junior Clinical Research Award from the American Cancer Society (grant JCRA-1).

Presented at the meeting of the American Laryngological Association, Los Angeles, California, April 17-18, 1993.

REPRINTS — Jay F. Piccirillo, MD, Dept of Otolaryngology, Box 8115, Washington University School of Medicine, 517 S Euclid Ave, St Louis, MO 63110.

the inclusion of symptom type, symptom severity, and comorbidity. Despite this work[36] and other work[37] outlining the deficiencies of the current staging system for cancers of the head and neck, strict adherence to a morphological staging system persists. The multiple reasons for this persistence include 1) belief in the exclusive importance of morphology, 2) disbelief in the reliability of patient-based variables, such as symptom type, duration, and severity, 3) previous absence of a taxonomy for classifying the clinical information, 4) desire to keep the staging system simple by including only one main variable — morphology, and 5) previous absence of statistical techniques and suitable computer capabilities for analyzing multiple variables (Piccirillo and Feinstein, unpublished observations).

In the current research we had two main purposes: first, to validate, in a more modern cohort of patients with laryngeal cancer, the previously demonstrated[37] prognostic importance of staging symptoms and comorbidity; and second, to create, with multivariable statistical procedures used to combine prognostically relevant clinical and morphological data, a new composite staging system that substantially improves the prognostic precision of TNM staging alone.

METHODS

The general methods for collecting and analyzing data in these epidemiologic assessments of cancer therapy have been reported elsewhere in detail[23-25] and in the earlier study of cancer of the larynx.[36] In the following sections we describe the current population under study, and the methods of data collection, classification, and analysis.

POPULATION UNDER STUDY

All patients first treated at Yale–New Haven Hospital (YNHH) between January 1, 1973, and December 31, 1985, for a primary carcinoma of the larynx were identified through the Yale Tumor Registry and diagnostic lists maintained by the Medical Records Department of YNHH. This time span was chosen to obtain a significant number of patients and also to allow collection of follow-up data for at least 5 years for each patient. The tumor registry identified 206 patients, all of whom had medical records available for analysis. Of these 206 patients, 10 were excluded from further analysis: 9 because the cancer was not squamous cell, and 1 because the cancer had no histologic proof.

The remaining 196 patients formed an inception cohort[38] with a morphologically acceptable, histologically documented primary squamous cell carcinoma of the larynx first treated at YNHH between 1973 and 1985. For all but 9 patients, full 5-year follow-up information was obtained from the tumor registry and medical records. The National Death Index[39] yielded sufficient follow-up information for 6 of the remaining 9 patients. Therefore, full 5-year follow-up was obtained for 193 patients. When needed for individual patients, additional information was obtained from other hospitals, consulting or referring physicians, the Connecticut State Tumor Registry, and the National Death Index.

COLLECTION OF DATA

A "zero time" was selected for each patient as the date of the first antineoplastic therapy directed at the laryngeal cancer. In patients who received no antineoplastic treatment, the date of the no-therapy decision was selected as zero time. If no such decision was made, the date of cancer diagnosis was chosen.

The information for each patient was extracted onto a special form. The data for the zero-time "baseline" state and pre-zero interval included basic demographic information; a history of symptom type, duration, and severity; a complete anatomic description of the primary tumor and nodal and distant metastatic spread, if known; a summary of other medical conditions; and results of pertinent laboratory and radiographic studies. The recorded details of therapy, follow-up data, and survival for the post–zero-time interval were also included.

CLASSIFICATION OF DATA

Because the archival data may contain certain imperfections, consistent methods of management are required. The general methods of management have been discussed elsewhere.[23-25,27]

The additional specific techniques used in this study were as follows.

Symptoms. Two main problems must be managed when symptoms are analyzed for a formal staging of cancer. The first problem is a discrepancy about the existence or duration of symptoms in the various recorded medical histories. We regarded a symptom as existing if it was mentioned in at least one of the recorded notes. When two or more possible time spans were cited for the date of first cancer symptoms, the longest duration of definite symptoms was selected.

The second problem occurs in decisions about attributing each symptom either to the cancer or to a coexistent disease. We attributed a symptom to the carcinoma if it was consistent with the cancer's manifestations and if no other disease was noted as a possible cause. The symptom was regarded as having uncertain attribution when the cause could be the cancer, some other identified disease, or both. For example, hemoptysis would have uncertain attribution in a patient with both carcinoma of the larynx and a primary lung cancer. A symptom was regarded as nonattributable when it was completely compatible with a comorbid cause. The patient's symptomatic state at zero time was classified according to symptoms that had either definite or uncertain attribution. Symptoms regarded as nonattributable were excluded from classification of symptom status, but may have been used for classifying severity of comorbid conditions.

Symptom Stages. After discussions with other otolaryngologists, and after exploring the prognostic effects of the various categories of symptoms alone or in combination, we classified the patient's symptoms into four symptomatic stages.

1. Local symptoms, which can be attributed to the tumor at its primary site. Examples are hoarseness and voice change.

2. Perilocal symptoms, which can be attributed to inflammation surrounding the tumor at its primary site. Examples are throat irritation, fullness, hemoptysis, and otalgia.

3. Extralocal symptoms, which can be attributed to the tumor's interference with normal function within the upper aerodigestive tract or in the body as a whole. Examples are respiratory difficulty, cough, dysphagia, odynophagia, malaise, fatigue, and significant weight loss (defined as nondeliberate loss of 10% or more from the patient's customary weight. If the percentage could not be calculated, a loss of 9 kg or more was accepted as significant). If weight loss was the patient's main complaint, it was accepted as significant even when absolute values or percentages of loss were slightly below the figures just cited.

4. Distant symptoms, which imply that the cancer has spread beyond the primary locus. Such symptoms must be accompanied by physical, radiographic, or other laboratory evidence to document regional or distant disease. Examples are neck lump, neck pain, and vision disturbance as part of Horner's syndrome.

ANATOMIC EXTENSIVENESS

The boundaries of the three anatomic subsites within the larynx — supraglottic, glottic, and subglottic — were defined by the American Joint Committee (AJC) for Cancer Staging.[10] The 1988 AJC TNM criteria were used for classifying the anatomic extensiveness of the tumor and stage groupings.

We had at first planned to use the TNM classification originally assigned to the patient by the attending physician at zero time. Such statements were often unavailable, however, and when available, were found to show significant discrepancies in the stages designated by the various physicians caring for each patient. We therefore decided that all tumors would be restaged for this project with the 1988 AJC criteria applied by the board-certified otolaryngologist (J.F.P.) who conducted the medical record review. All pre–zero-time data, including results of the direct laryngoscopy and radiographic studies, were used for staging, but we did not use any evidence obtained after zero time. Additional tumor data obtained at the time of primary surgical treatment were not incorporated into the pretreatment staging system. Furthermore, pre–zero-time data were not modified as a result of information derived from post–zero-time surgical specimens, even if the post–zero-time data may have disagreed with the pre–zero-time data. (The creation of a post–zero-time staging system, utilizing information obtained from initial treatment, will be the focus of a subsequent paper.)

Discrepancies in Symptom History and Anatomic Description. During medical record review, symptom history and anatomic description were recorded for each patient. Discrepancies among reporting physicians were classified into four categories according to the effect on final classification. The categories of discrepancy were 1) none, 2) within one stage, 3) across one stage, and 4) across two or more stages.

Histopathology. When available, the grade of the primary tumor was recorded for all patients, and was cited from the biopsy specimen for those who received radiotherapy only. Primary tumor histology was recorded from the primary surgical resection rather than from the biopsy specimen for patients who initially received surgical therapy. When more than one grade was noted from a single specimen, the most anaplastic description was cited.

Prognostic Comorbidity. The presence of associated diseases, which are usually nonneoplastic and not related to the cancer, are called comorbidities. They have been found[26] to have important effects on prognosis and treatment. In this study we used the term prognostic comorbidity for concomitant ailments that could significantly reduce the patient's prognostic anticipations for life. Such ailments would be classified as grade 3 (severe) organ decompensation in the Kaplan-Feinstein classification system.[40] Examples of such ailments are recent myocardial infarction, far-advanced tuberculosis, recent severe stroke, severe liver disease, severe lung disease, and uremia. Examples of grade 2 (moderate) organ decompensation comorbidity include congestive heart failure more than 6 months previously, moderate pulmonary insufficiency (ie, dyspnea on slight exertion), old stroke with residua, recent acute pancreatitis, and recurrent hospitalizations for alcohol-related ailments such as gastritis or pancreatitis. Examples of grade 1 (mild) organ decompensation comorbidity include electrocardiographic evidence of coronary disease or atrial fibrillation, old stroke without residua, recent active tuberculosis, recurrent lower urinary tract infections or renal stones, and intermittent claudication.

Therapeutic Nil Hypothesis. To combine all patients' data for identifying prognostic factors and creating staging systems, each patient's classification at zero time was analyzed for the eventual outcome, regardless of the intervening treatment. The combination of all types of therapy according to this therapeutic "nil hypothesis" is discussed elsewhere.[41-44]

Follow-up. Each patient's post–zero-time course was extracted and coded to cite whatever clinical, anatomic (recurrence and new primary cancer), and therapeutic changes had occurred.

Outcome. Although information on tumor recurrence and tumor status at death (or last follow-up) was extracted, 5-year survival status was selected as the primary outcome measure because of its standard use in prognostic studies of patients with cancer.

DATA ANALYSIS

After all medical record data were transferred to coding forms, the computerized data entry was done by a professional data management company and subjected to double entry and verification. The medical record extraction and code forms were used for spot checks of the entered data. Sorting, tabulation, and statistical analysis, including multivariable analyses, were done with the Statistical Analysis Software system (SAS Institute Inc, Cary, NC). An additional multivariable approach, called conjunctive consolidation,[29] was used to combine prognostically important variables.

RESULTS

BASELINE CLASSIFICATION AND 5-YEAR SURVIVAL RESULTS

The cohort of 193 patients contained 151 men and 42 women, with 168 patients being white, 24 African-American, 3 Hispanic, and 1 Asian. The median age was 62 years, with 52 (27%) patients being 52 years of age or younger, 96 (50%) between 53 and 67, and 45 (23%) older than 67 years.

Table 1 shows the 5-year survival results according to eight baseline variables of classification. Gender had little prognostic effect, but 5-year survival progressively declined with age. The results for cigarette smoking showed an unexpected trend, with survival increasing as smoking increased, but it was not statistically significant. As expected, survival rates decreased with higher TNM stages and with decreasing grades of histopathologic differentiation, but were surprisingly higher in patients with long rather than short durations of symptoms. The survival gradients for histologic grade and symptom duration, however, were not statistically significant. As expected, symptom stage and prognostic comorbidity had major impacts on survival. Because age is a demographic rather than a clinical variable, the composite staging systems described later were constructed only from symptom stage, TNM stage, and prognostic comorbidity.

DISCREPANCY IN REPORTING OF SYMPTOMS AND ANATOMY

Symptoms are usually regarded as subjective data, too unreliable and inaccurate for use in clinical research or practice, and anatomic information is usually perceived as much more objective and accurate. To evaluate this belief, we compared the discrepancies when physicians reported a patient's symptoms versus when they described anatomic stage of the tumor.

As shown in Table 2, discrepancies did not occur for symptom stage in 179 patients, for anatomic stage in 127 patients, or for either in 117 patients. Anatomic discrepancies occurred within one stage for 22 patients, across one stage for 42, and across two stages for 2. In contrast, discrepancies in symptom history were present only within one stage for 4 patients, across one stage for 7, and across two stages for 3. The results confirm previous studies of inconsistency in anatomic staging[45,46] and suggest that symptom staging is at least as reliable as anatomic staging.

SURVIVAL IN COMBINED SYMPTOMATIC–TNM ANATOMIC CATEGORIES

To demonstrate that symptoms offer separate, distinctive prognostic information, the conjoined effects of symptom and TNM stages are shown in Table 3. Because the marginal totals for the rows and columns previously appeared in Table 1, the important information of Table 3 is in the cells. Within each TNM stage, the symptom stages produce a distinctive prognostic gradient (except in cells with small numbers of patients). For example, for TNM stage I, the gradient goes from 83% to 50% from symptom stage 1 to symptom stage 3, and correspondingly, in TNM stage II, from 80% to 44%.

TABLE 1. FIVE-YEAR SURVIVAL RATES ACCORDING TO EIGHT BASELINE VARIABLES OF CLASSIFICATION

		Number			
Variables	*Categories*	*Patients*	*Survivors*	*Percent*	*Survival Gradient**
Total population		193	127	66	
Gender	Women	42	29	69	4 (NS)
	Men	151	98	65	
Age	≤52	52	42	81	
	53-67	96	66	69	39 ($p < .0001$)
	≥68	45	19	42	
Cigarette smoking†	>2 pks/d	51	38	74	
	≤2 pks/d	125	80	64	21 (NS)
	Never	15	8	53	
TNM stage	I	77	60	78	
	II	48	32	67	
	III	45	27	60	43 ($p < .001$)
	IV	23	8	35	
Histopathology grade	Carcinoma in situ (includes microinvasion)	13	10	77	
	Well	79	55	70	
	Moderate	35	22	63	
	Poor	22	10	45	32 (NS)
	Other‡	5	3	60	
	Unknown	39	27	69	
Symptom duration	0-6 mo	101	61	60	
	>6 mo	92	66	72	12 (NS)
Symptom stage	Local§ (1)	81	65	80	
	Perilocal (2)	34	26	76	
	Extralocal (3)	62	30	48	42 ($p < .0001$)
	Distant (4)	16	6	38	
Prognostic comorbidity	Absent	166	123	74	
	Present	27	4	15	59 ($p < .0001$)

χ^2 Used for linear trend where appropriate. Significance levels given by χ^2 test.

NS — not significant.

*Gradient represents difference in survival rates between highest and lowest rates in each category.

†Baseline data unknown for 2 patients.

‡Other includes verrucous ($n = 3$) and anaplastic ($n = 2$).

§Includes 2 asymptomatic patients; 1 was 5-year survivor.

If symptoms merely reflected anatomy, the small, localized tumors of stage I would produce only mild symptoms, while large, extensive tumors would give severe symptoms. Nevertheless, as shown by the denominators in each cell of Table 3, many patients with small tumors had severe symptoms, and those with large tumors often had mild symptoms.

SURVIVAL IN COMBINED SYMPTOMATIC-PROGNOSTIC COMORBIDITY

Categories of Functional Severity. Table 4 shows the prognostic impact of comorbidity within each symptom stage. In each category of symptom stage, the survival rates were substantially lowered when prognostic comorbidity was present. Because both the symptom severity and prognostic comorbidity stages were distinctively important, we combined the categories, using the conjunctive consolidation strategy described elsewhere.[47-49] The basis of the strategy is to join groups that are biologically coherent and that have statistical isometry, ie, reasonably similar survival rates.[29] Thus, the eight comorbidity-symptomatic groups were consolidated, as shown in Table 4, into three composite stages labeled as alpha, beta, and gamma. Patients with prognostic comorbidity, regardless of symptom stage, formed the gamma group. Among the patients without prognostic comorbidity, the beta group was formed by symptom stages 3 and 4, and the alpha group, by symptom stages 1 and 2.

Because symptoms and prognostic comorbidity indicate the patient's functional status, the new composite categories were called the functional severity

TABLE 2. DISTRIBUTION OF PATIENTS BY DISCREPANCY IN SYMPTOMS AND ANATOMIC REPORT

	Anatomic Discrepancy				
Symptom Discrepancy	*None*	*Within One Stage*	*Across One Stage*	*Across Two Stages*	*Total*
None	117	20	40	2	179
Within one stage	4	0	0	0	4
Across one stage	5	1	1	0	7
Across two stages	1	1	1	0	3
Total	127	22	42	2	193

(FS) stages. Their results are shown in the right-hand marginal totals of Table 5. In the three composite categories of FS, the 5-year survival rates were as follows: alpha, 89/107 (83%); beta, 34/59 (58%); and gamma, 4/27 (15%). The results are statistically significant by χ^2 test between adjacent stages and show a χ^2 for linear trend of 47.3, with $p < .0001$.

CONJUNCTION OF FUNCTIONAL SEVERITY WITH ANATOMIC STAGING

If the cancer's morphology and the patient's functional state were perfectly correlated, the FS staging system would be prognostically unnecessary: the TNM anatomic stage would provide all the important prognostic information. The frequent dissociation between form and function, however, is demonstrated by the results of Table 5 for the conjunction of FS and TNM anatomic stages.

Within each vertical column of TNM anatomic stages, the FS staging system demarcates an important and consistent prognostic gradient. It extends from 88% to 17% for 5-year survival rates within TNM stage I, from 80% to 14% within TNM stage II, from 69% to 28% in stage III, and from 75% to 0% in stage IV. The results show the profound prognostic heterogeneity that can exist among patients who are in the same TNM stage.

TABLE 3. FIVE-YEAR SURVIVAL RATES IN CONJUNCTION OF SYMPTOM AND ANATOMIC STAGES

Symptom Stage	*TNM Anatomic Stage* *I*	*II*	*III*	*IV*	*Total*
1	43/52 (83%)	16/20 (80%)	5/8 (62%)	1/1 (100%)	65/81 (80%)
2	11/14 (78%)	8/10 (80%)	5/7 (71%)	2/3 (67%)	26/34 (76%)
3	5/10 (50%)	8/18 (44%)	15/26 (58%)	2/8 (25%)	30/62 (48%)
4	1/1 (100%)	0 (0%)	2/4 (50%)	3/11 (27%)	6/16 (38%)
Total	60/77 (78%)	32/48 (67%)	27/45 (60%)	8/23 (35%)	127/193 (66%)

Denominators — number of patients in each category, numerators — corresponding number of 5-year survivors.

TABLE 4. FIVE-YEAR SURVIVAL RATES IN CONJUNCTIVE CONSOLIDATION OF SYMPTOM SEVERITY AND PROGNOSTIC COMORBIDITY STAGES

Symptom Stage		*Prognostic Comorbidity Stage* *Absent*		*Present*	*Total*
1	Alpha	64/76 (84%)	Gamma	1/5 (20%)	65/81 (80%)
2		25/31 (81%)		1/3 (33%)	26/34 (76%)
3	Beta	28/47 (60%)		2/15 (13%)	30/62 (48%)
4		6/12 (50%)		0/4 (0%)	6/16 (38%)
Total		123/166 (74%)		4/27 (15%)	127/193 (66%)

CONSOLIDATION OF FUNCTIONAL SEVERITY AND TNM ANATOMIC STAGING

Although the main point of the research was to demonstrate the prognostic importance of FS, another prime goal was to allow FS to be incorporated into TNM staging. The easiest mechanism to achieve that goal was another conjunctive consolidation, joining appropriate categories of the two staging systems. The pattern of consolidation and the new names for the four-category composite clinical severity (CS) staging system are shown in Table 6. The union of the two worst stages, TNM anatomic stage IV and FS stage gamma, formed composite CS stage D. The union of the remaining two next-worst stages, TNM anatomic stage III and FS stage beta, formed CS stage C. The remaining patients in FS alpha were then divided, with TNM anatomic stage II forming CS stage B and TNM anatomic stage I forming CS stage A. The latter two-part division of FS stage alpha depended on clinical judgment rather than statistics alone, because the alpha group patients in TNM

TABLE 5. FIVE-YEAR SURVIVAL RATES IN CONJUNCTION OF FUNCTIONAL SEVERITY AND TNM ANATOMIC STAGES

Functional Severity Stage	*TNM Anatomic Stage* *I*	*II*	*III*	*IV*	*Total*
Alpha	53/60 (88%)	24/30 (80%)	9/13 (69%)	3/4 (75%)	89/107 (83%)
Beta	6/11 (55%)	7/11 (64%)	16/25 (64%)	5/12 (42%)	34/59 (58%)
Gamma	1/6 (17%)	1/7 (14%)	2/7 (28%)	0/7 (0%)	4/27 (15%)
Total	60/77 (78%)	32/48 (67%)	27/45 (60%)	8/23 (35%)	127/193 (66%)

TABLE 6. PATTERN OF CONSOLIDATION OF FUNCTIONAL SEVERITY AND TNM ANATOMIC STAGES TO FORM COMPOSITE CLINICAL SEVERITY STAGING SYSTEM

Functional Severity Stage	*TNM Anatomic Stage* I	*II*	*III*	*IV*
Alpha	A	B	C	D
Beta	C	C	C	D
Gamma	D	D	D	D

anatomic stages I and II had only a relatively small gradient (8%) in survival rates.

The results of the new composite CS staging system are shown in Table 7. The overall survival gradient is 60%; the gradients are significantly different between stages B and C, and C and D, and the χ^2 for linear trend is 30.47 with $p < .0001$.

MULTIVARIABLE ANALYSES WITH CONVENTIONAL ALGEBRAIC METHODS

Although conjunctive consolidation is a simple, effective, and easy to understand method of creating a multivariable staging system, the conventional form of statistical analysis for the data here would be logistic regression. The independent variables would be the four baseline variables (age, TNM stage, symptom stage, and prognostic comorbidity) that seemed to have important effects on 5-year survival in Table 1.

Three different arrangements of independent variables were analyzed in both a forward- and backward-stepping model. In arrangement 1, the four baseline clinical variables were all entered in their elemental state. In arrangement 2, the FS staging system formed from the two clinical variables, symptom stage and comorbidity, was entered together with age and TNM anatomic stage. In arrangement 3, the composite CS staging system, formed by the consolidation of symptom stage, comorbidity, and TNM anatomic stage, and age were entered. The forward-stepping logistic regression results and rankings of variables for the three arrangements are shown in Table 8.

Regardless of which arrangement was used, the results confirmed the fundamental importance of prognostic comorbidity and symptom stage, appearing alone or in the composite stages. Age was the only other baseline independent variable that had a statistically significant relationship to outcome. Surprisingly, TNM anatomic stage was not found to be statistically significant in either of the two arrangements in which symptom stage and comorbidity also appeared as candidate variables in logistic regression.

TABLE 7. FIVE-YEAR SURVIVAL RATES IN COMPOSITE CLINICAL SEVERITY STAGING SYSTEM

Stage	*Five-Year Survival*
A	53/60 (88%)
B	24/30 (80%)
C	38/60 (63%)
D	12/43 (28%)
Total	127/193 (66%)

Overall survival gradient is 60%.

EFFECT OF AGE

Despite its demonstrated survival importance, age was not included in the composite CS system, mainly because we wanted the staging system to use clinical rather than demographic variables. Three other reasons, however, were that age in an older population (the median age of patients with cancer of the larynx is 62) may simply have an actuarial survival effect; age may also correlate with the subsequent new comorbidity that occurs in older patients during their follow-up period, after zero time; and finally, because the CS staging system cannot perfectly capture all aspects of prognosis, age may be a surrogate for another severity factor, such as poor nutritional status[50] or performance status,[51] that was not identified and included in the current research.

QUANTITATIVE EVALUATION OF STAGING SYSTEMS

The purpose of staging is to divide a large group into smaller subgroups that are internally homogeneous and externally disparate. Although the comparison of different staging systems is best done for face validity or common sense, certain statistical scores and tests can be used for quantitative evaluation of each system's mathematical accomplishments of the system. The evaluation principles, which have been discussed in detail elsewhere,[41-44] were applied to results of three staging systems for the 193 patients with laryngeal cancer: the standard 1988 AJC TNM criteria, the new FS system, and the composite CS system that combines the TNM and FS systems.

As shown in Table 9, all three systems had a monotonic survival gradient. In every other feature, however, the FS and CS systems were quantitatively superior to the TNM system. The FS and CS systems were tied at 29% for proportionate reduction in predictive errors, but otherwise the FS system had higher quantitative achievements than did the CS system.

DISCUSSION

Despite many articles[11,13,14] demonstrating the shortcomings of the AJC TNM staging system, patients with cancer continue to be described by the

TABLE 8. RESULTS OF LOGISTIC REGRESSION: MULTIVARIATE ANALYSIS OF VARIABLES FOR STAGING

	Arrangement 1			*Arrangement 2*			*Arrangement 3*		
Variable	χ^2	*p*	*Rank*	χ^2	*p*	*Rank*	χ^2	*p*	*Rank*
Prognostic comorbidity	34.30	.0001	1		Not included			Not included	
Symptom stage	13.08	.0003	2		Not included			Not included	
Age	10.25	.0014	3	8.45	.0037	2	13.13	.0003	2
Functional severity stage		Not included		44.10	.0001	1		Not included	
TNM stage	1.51	.2190	4	3.09	.0783	3		Not included	
Clinical severity stage		Not included			Not included		38.21	.0001	1

morphology of the tumor, and little or no attention is given to the importance of the associated clinical manifestations.

The idea of using symptoms as a prognostic guide in cancer is not new. In 1961, for patients with cancer of the larynx, Jackson and Norris[52] mentioned the prognostic importance of such "late" symptoms as "... increasing degrees of dyspnea, stridor, hoarseness and dysphagia, with persistent pain on swallowing and progressive cachexia." Taylor et al[34] noted, in a multi-institutional study of patients with osteosarcoma, that duration of symptoms, weight loss, and swelling at the primary site provided prognostic information supplementary to the morphological description of the tumor. Neel et al[35] demonstrated that in addition to extent of tumor, prognosis for patients with nasopharyngeal carcinoma was related to duration of symptoms and the total number of symptoms at the time of diagnosis. Both in the current research and the previous study of cancer of the larynx,[36] symptom severity offered additional prognostic information not available from classifying anatomy alone. The advantage of symptomatic staging, therefore, is that symptoms correlate reasonably well with the intralaryngeal anatomy, while adding important prognostic dimensions that cannot be discerned from anatomy alone. Because the currently available nonsurgical diagnostic procedures do not establish the true extent of the disease, and because morphological information does not indicate the virulence or rate of tumor growth, symptom severity provides an index of biologic behavior that cannot be otherwise identified. Comorbidity also provides additional prognostic information for long-term survival.

TABLE 9. QUANTITATIVE EVALUATION OF STAGING SYSTEMS

	Staging Systems		
Category of Evaluation	*TNM Anatomic (1988)*	*Functional Severity*	*Clinical Severity*
Monotonicity of survival gradient	Yes	Yes	Yes
Range of overall survival gradient	43%	68%	60%
Proportionate reduction in predictive errors	11%	29%	29%
Proportionate reduction in variance	0.094	0.240	0.219
χ^2 For linear trend	15.55	47.30	30.47

The FS classification of symptom type and comorbidity is a powerful staging system that could predict 5-year survival more effectively than the TNM anatomic system, and it has two main attractions for clinical use: 1) it requires no special technologic information and should be relatively stable despite changing technologic methods of anatomic diagnoses[53]; and 2) it can be applied by any health care professional using readily available information from the patient's history.

Nevertheless, because we wanted to augment rather than eliminate the TNM system, we combined the FS stages and TNM anatomic stages to form a composite CS system, which is more prognostically precise than TNM anatomic staging alone.

The following three reasons may explain why the TNM anatomic system did not prognosticate better.

1. When compared with suitable clinical descriptions of a cancer patient, the morphology of the tumor may in fact have lesser prognostic importance.

2. The current TNM system may not express the true prognostic impact of morphology.

3. The retrospective extraction of the morphological data from archival records may have led to unavoidable inaccuracies.

Regardless of why the TNM system is imperfect, however, the particular symptom-stage groupings developed in this research paper can be regarded as an additional, though not final, step in the scientific process of quantifying the clinical manifestations of cancer of the larynx. The consistency of results for various cancer studies at various times serves as validation for the general clinical (rather than purely morphological) principles of cancer staging. To validate these principles prospectively, the first author has begun working to collect this important information from other physicians specializing in the care of patients with head and neck cancer.

Using the new staging system and data collected for the current project, we are evaluating the effectiveness of current treatments, possible predictors for the development of recurrence, the prognostic impact of post-therapeutic variables (such as surgical margins, complications, and pathologic lymph node status), and the impact of blood transfusions. We are also analyzing the results by using other measures, such as quality of life, for the dependent outcome measures.

CONCLUSIONS

Because current anatomic staging systems identify form without identifying function, the inclusion of clinical variables in a formal staging system can strikingly improve prognostic accuracy. The scientific evaluation of treatment and the care of patients are currently impeded by the rigid adherence to a staging system based solely on morphological descriptions of the cancer while ignoring suitable descriptions of the patient.

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LARYNGEAL BEHAVIOR IN UNILATERAL SUPERIOR LARYNGEAL NERVE PARALYSIS

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Laryngeal behavior in unilateral superior laryngeal nerve (SLN) paralysis was investigated in animal models and clinical cases. The occurrence of an oblique glottis caused by rotation of the posterior glottis to the paralytic side was the main focus of this study. The animal model study employed live dogs. When the SLN on one side was sectioned, spontaneous phonation did not cause a significantly oblique glottis. When the unaffected SLN was electrically stimulated during spontaneous phonation, an oblique glottis occurred. When the SLN was unilaterally stimulated during spontaneous phonation with both SLNs sectioned, a markedly oblique glottis occurred. In the clinical study, larynges of 17 patients with SLN paralysis were examined during a test task in which a low-pitched phonation was followed by a high-pitched phonation. The purpose of this task was to activate the unaffected cricothyroid muscle during the test phonation. Five patients could not perform the test task. The glottis obviously rotated in 9 patients, whereas no significant rotation of the glottis occurred in 3. One of the latter 3 had an incomplete paralysis, and the other 2 had marked scarring around the laryngeal framework. We conclude that a unilateral SLN paralysis causes a rotation of the posterior glottis to the paralytic side when the unaffected cricothyroid muscle is markedly activated. The test consisting of low-pitched phonation followed by high-pitched phonation is a relatively simple and accurate diagnostic procedure for unilateral SLN paralysis.

KEY WORDS — cricothyroid muscle, oblique glottis, superior laryngeal nerve, superior laryngeal nerve paralysis.

INTRODUCTION

Is there any characteristic behavior of the larynx in unilateral superior laryngeal nerve (SLN) paralysis? This question has been argued for more than 80 years. Since Mygind[1] reported in 1906 that the glottis was oblique during phonation in patients with unilateral SLN paralysis, several laryngologists[2-5] have stated that an oblique glottis is one of the most manifest findings in those patients and that observation of the laryngeal behavior is useful for diagnosis of unilateral SLN paralysis. Other laryngologists,[6-8] however, rarely noticed an oblique glottis in their patients and neglected the clinical utility of this finding. Also, in experiments on section or stimulation of the SLN,[7,9-11] there have been conflicting results on the occurrence of an oblique glottis.

Arnold[12] explained the mechanism of the oblique glottis in unilateral SLN paralysis. According to his interpretation, a contraction of the cricothyroid (CT) muscle on the unaffected side causes rotation of the cricoid cartilage against the thyroid cartilage and moves the posterior glottis to the paralytic side. If this mechanism actually works, the degree of obliquity of the glottis should depend on the strength of contraction of the CT muscle. In other words, the oblique glottis may not be noticed when the CT muscle contracts weakly. An oblique glottis should be consistently manifested only when the CT muscle contracts strongly. Differences in the degree of CT muscle contraction are inferred to be the reason why there have been conflicting results on the occurrence of an oblique glottis in unilateral SLN paralysis in the clinical and experimental studies previously reported.

The purposes of this study were to investigate laryngeal behavior, especially the occurrence of an oblique glottis caused by a rotation of the posterior glottis to the paralytic side, in SLN paralysis both in animal models and clinical cases and to propose a diagnostic procedure for SLN paralysis.

MATERIALS, SUBJECTS, AND METHODS

Animal Models. Three mongrel adult dogs were used. In each dog, the following experiments were conducted.

The animal was fixed in the supine position with the neck extended under general anesthesia with an intramuscular injection of ketamine hydrochloride (15 mg/kg). Subdermal local anesthetic with 0.5% mepivacaine hydrochloride was given in the anterior neck. Following skin incision, the larynx and trachea were exposed with the strap muscles sectioned. The bilateral external branches of the SLN were identified carefully.

In the first experiment (model 1), the left SLN was

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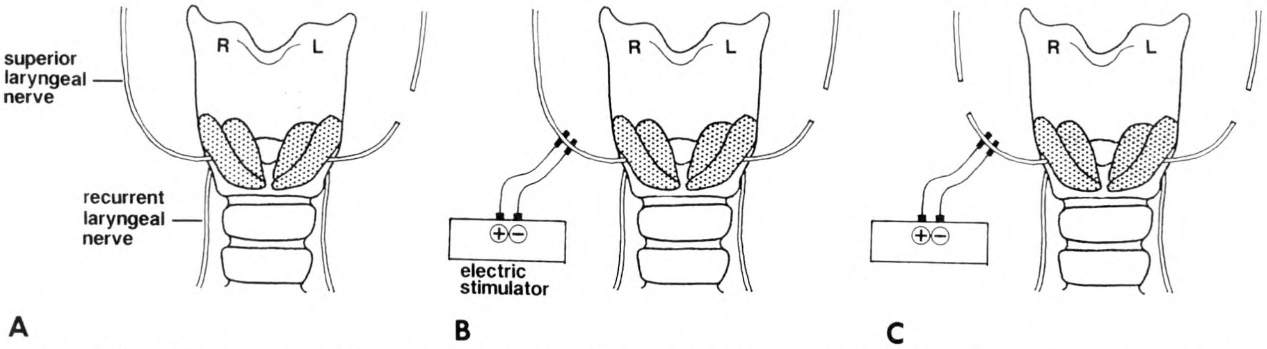

Fig 1. Schematic presentation of three animal models. A) Model 1, in which left superior laryngeal nerve (SLN) was sectioned. B) Model 2, in which left SLN was sectioned and right SLN was electrically stimulated. C) Model 3, in which bilateral SLNs were sectioned and right SLN was electrically stimulated.

sectioned 2 cm from the CT muscle (Fig 1A). When the level of anesthesia became light, the animals phonated spontaneously.

In the second experiment (model 2), the right SLN was electrically stimulated shortly after spontaneous phonation started; the left SLN had been sectioned (Fig 1B). The frequency, duration, and amplitude of the electrical stimulation were 30 Hz, 1 millisecond, and 1 V, respectively. In this model, additional activation was provided to the right CT muscle during spontaneous phonation.

In the third experiment (model 3), both SLNs were sectioned. During spontaneous phonation, the right SLN was electrically stimulated in the same way as in the second model (Fig 1C). In this model, strong activation was added to the right CT muscle, which had no contraction during spontaneous phonation.

In all three models, the left CT muscle was inactive. The activity of the right CT muscle was less in model 1 than in models 2 and 3. The difference in right CT muscle activity between before and during electrical stimulation was greater in model 3 than in model 2.

Images of the larynx viewed with a transoral fiberscope were recorded on videotape during the experiments. The images of the larynx and audio signals on video were closely reviewed relative to glottic rotation and changes in pitch.

Clinical Study. During the period from January 1991 to June 1992, the activity of the CT muscle in 40 patients with voice disorders was examined with electromyography in Kurume University Hospital. Of the 40, 17 were found by electromyography to have unilateral SLN paralysis, because a unilateral CT muscle presented with no or markedly reduced voluntary activity during high-pitched phonation.

Of these 17 patients, 8 had a unilateral high vagal nerve paralysis accompanied with an immobile soft palate on the affected side, 5 had a unilateral SLN paralysis accompanied by ipsilateral recurrent laryngeal nerve (RLN) paralysis, and 4 had a unilateral SLN paralysis without any other nerve involvement.

The laryngeal behavior was recorded on video by means of a fiberscope when a low-pitched phonation was followed by a high-pitched phonation. An occurrence of a glottic rotation was evaluated on video images by three well-qualified laryngologists.

RESULTS

Animal Models. In all three animals, similar results were obtained for each model.

Model 1 involved section of the left SLN. The bilateral vocal folds presented with nearly symmetric movements, and the unilateral SLN paralysis was not manifested in laryngeal behavior during phonation (Fig 2).

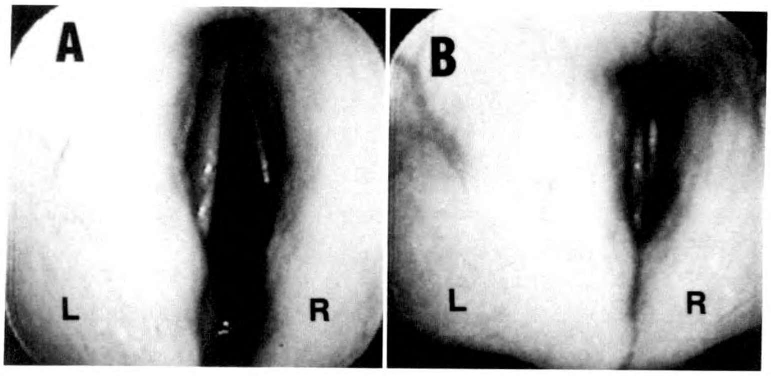

Fig 2. Laryngeal images in animal model 1. A) Before phonation. B) During phonation (fundamental frequency [F0] of 240 Hz).

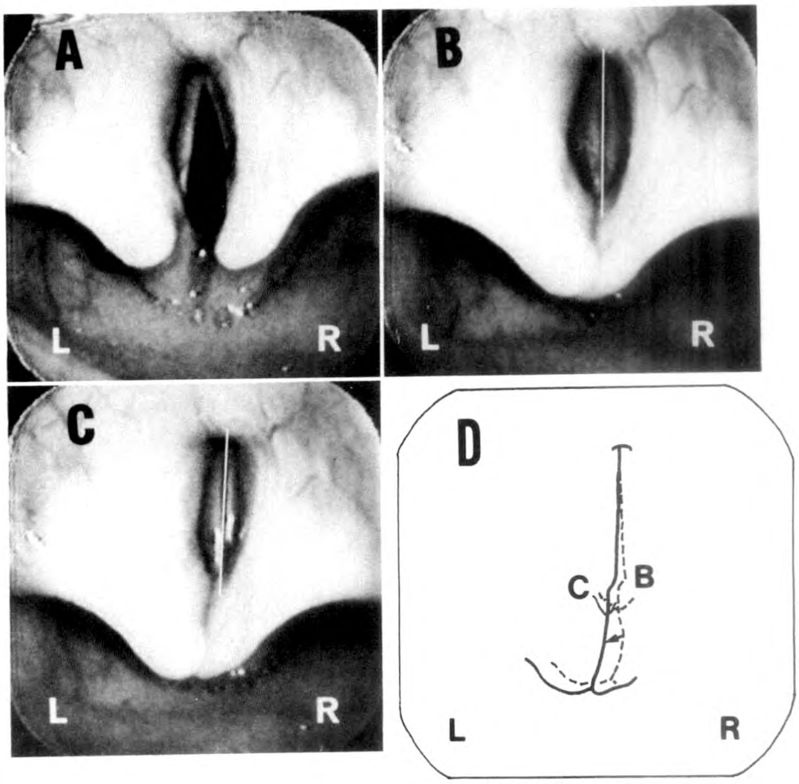

Fig 3. Laryngeal images in animal model 2. A) Before phonation. B) During spontaneous phonation (F0 = 250 Hz). C) During electrical stimulation (F0 = 500 Hz). D) Schematic drawing of glottic images in B and C superimposed. White line in B and C indicates axis of glottis. Arrow in D indicates rotation of posterior glottis for high pitch.

Model 2 involved section of the left SLN and electrical stimulation of the right SLN. The posterior glottis rotated to the left side when the right SLN was electrically stimulated shortly after spontaneous phonation (Fig 3). An elevation of the vocal pitch also took place during the SLN stimulation. The fundamental frequency (F0) during stimulation was nearly twice as high as that before stimulation.

Model 3 involved section of the bilateral SLN and electrical stimulation of the right SLN. A marked rotation of the posterior glottis to the left side oc-

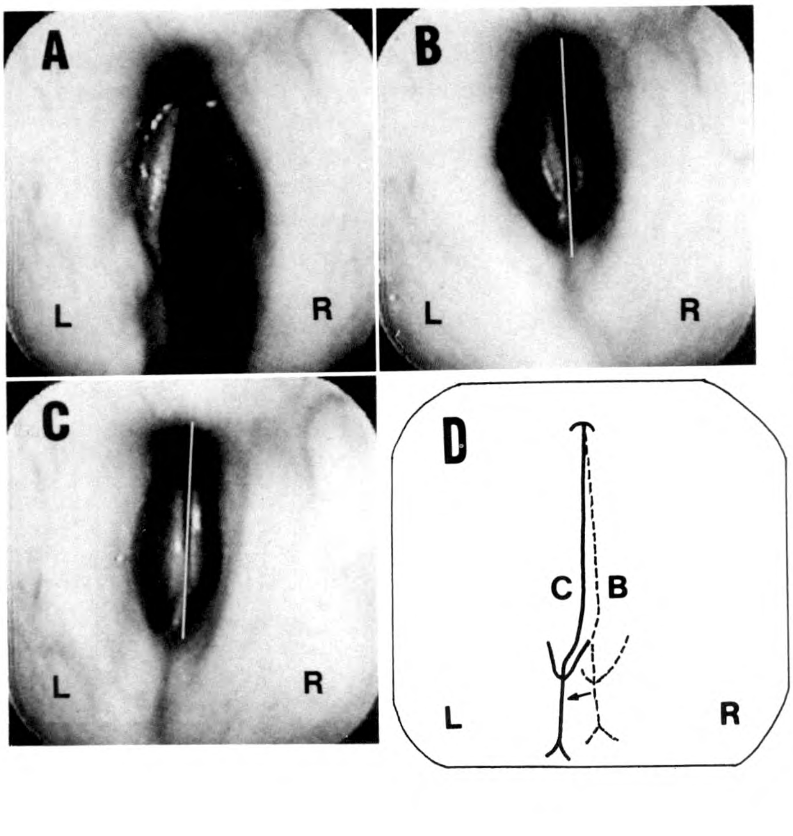

Fig 4. Laryngeal images in animal model 3. A) Before phonation. B) During spontaneous phonation before stimulation (F0 = 170 Hz). C) During electrical stimulation (F0 = 480 Hz). D) Schematic drawing of glottic images in B and C superimposed. White line in B and C indicates axis of glottis. Arrow in D indicates rotation of posterior glottis for high pitch.

SUMMARY OF TWELVE PATIENTS WITH UNILATERAL SUPERIOR LARYNGEAL NERVE PARALYSIS

Case	*Sex*	*Age (y)*	*Cause*	*Side*	*Activity of CT*	*Pitch Range in Semitones*	*Glottic Rotation During Pitch Elevation*
1*	M	51	Wallenberg's syndrome	Right	None	30	Obvious
2*	F	49	Neuritis	Right	Reduced	22	Not significant
3*	M	25	Cranial surgery	Left	None	35	Obvious
4*	F	75	Polyneuritis	Right	Reduced	17	Obvious
5*	F	71	Cranial surgery	Right	None	13	Obvious
6†	F	46	Unknown	Left	Reduced	24	Obvious
7†	F	66	Thyroid surgery	Left	None	14	Not significant
8†	F	44	Thyroid surgery	Right	None	24	Obvious
9†	F	41	Thyroid surgery	Left	None	14	Obvious
10	F	51	Thyroid surgery	Right	None	15	Not significant
11	F	54	Thyroid surgery	Left	Reduced	23	Obvious
12	F	68	Thyroid surgery	Left	None	11	Obvious

CT — cricothyroid muscle.

*High vagal nerve paralysis.

†Accompanied by recurrent laryngeal nerve paralysis.

curred at the time of nerve stimulation during phonation (Fig 4). Before stimulation, the vocal pitch was much lower than that during the spontaneous phonation of models 1 and 2. During stimulation, the pitch became as high as that during stimulation in model 2. The F0 during stimulation was almost three times as high as that before stimulation.

Clinical Study. Five of the 17 patients with unilateral SLN paralysis were not able to perform the test task, ie, production of a low-pitched voice followed by a high-pitched phonation, during laryngeal videofiberscopy. Test results, therefore, were obtained from 12 patients. The Table shows a summary of the 12 patients with SLN paralysis who could perform the test task during fiberscopy. Laryngeal images of a patient with right SLN and RLN paralysis are presented in Fig 5, demonstrating a marked glottic rotation.

In unilateral upper vagal nerve paralysis, 4 of 5 patients presented with an obvious rotation of the posterior glottis to the paralytic side during pitch elevation, whereas the fifth patient showed no significant rotation. The SLN paralysis of the latter was due to a neuritis accompanied by a common cold. The hoarseness had been improving at the time of examination, and electromyography of the CT muscle showed reduced activity. Therefore, this was a case of an incomplete paralysis during the course of recovery.

Of the 4 patients with unilateral SLN paralysis accompanied by RLN paralysis, 3 presented a marked rotation of the posterior glottis to the paralytic side, while the other had no significant rotation. In the latter, there was a remarkable scar around the larynx caused by extensive removal of the thyroid gland and the strap muscles. In 2 of the thyroid surgery patients who showed glottic rotation (Nos. 8 and 9 in the Table), the strap muscles were conserved and there was no significant scar in the anterior neck.

In the three cases of unilateral SLN paralysis

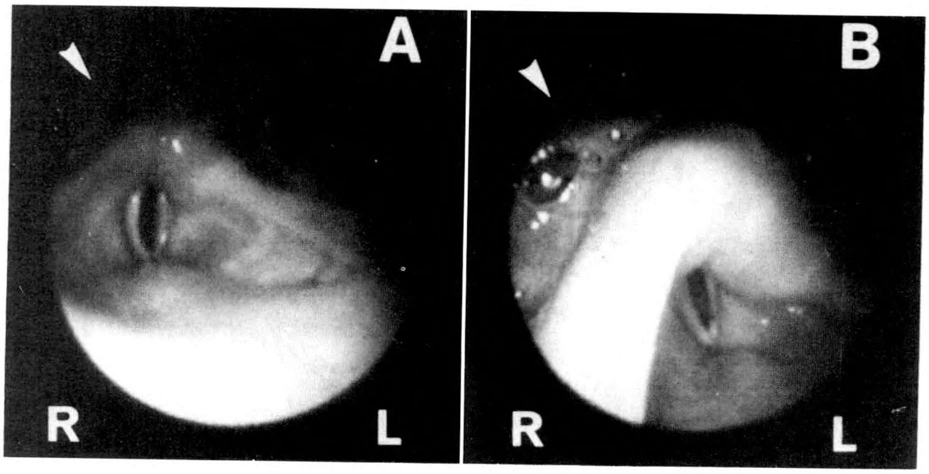

Fig 5. Laryngeal images of patient with right SLN paralysis. A) During phonation at low pitch (F0 = 180 Hz). B) During phonation at high pitch (F0 = 280 Hz). Arrowhead indicates location of 12 o'clock (reference point) of fiberscopic image. Note rotation of posterior glottis to right.

without RLN paralysis, the paralysis was caused by thyroid surgery. Two patients showed an obvious rotation of the posterior glottis to the paralytic side during pitch elevation, while the other had no significant rotation. A simple hemithyroidectomy was performed in the former two, and a wide removal including the strap muscles was conducted in the latter. A marked scar had formed around the larynx in the patient with no significant glottic rotation.

As a whole, in 9 of 12 patients with unilateral SLN paralysis, the posterior glottis obviously rotated to the paralytic side during pitch elevation. The other 3 patients, who showed no significant glottic rotation, had an incomplete paralysis during a course of recovery or marked scar formation around the larynx.

The pitch range of the 12 patients varied. It was not related to the CT muscle activity on the paralytic side or the occurrence of an oblique glottis.

DISCUSSION

It has been reported by several researchers[2-5] that the glottis becomes oblique during phonation in patients with unilateral SLN paralysis, since Mygind[1] reported 17 patients showing an oblique glottis. However, Gregg[6] stated in his clinical study examining hundreds of vocal folds with a laryngeal mirror that it was impossible to tell whether or not an SLN paralysis was present. Dedo[7] indicated that only 3 of 27 patients with unilateral SLN and RLN paralysis presented with the posterior larynx rotated to the paralytic side. In experiments of sectioning the unilateral SLN, some researchers[9,11] recognized an oblique glottis during phonation, whereas others[7] reported that little or no visible change occurred in vocal fold movement.

Why did these conflicting reports appear? The major reason may be that the degree of CT muscle activity on the unaffected side during examination was different among patients in clinical studies and among the animals in experimental studies. There was no description of the details of the phonation tasks or the vocal pitch in these previous reports. One can presume that in those reports in which an oblique glottis due to rotation of the posterior glottis to the paralytic side was obvious, the phonation was associated with a strong contralateral CT muscle activity, while the contralateral CT activity was low in those studies in which no significant glottic rotation was noticed during phonation.

In the animal models of the present study, after sectioning a unilateral SLN, an additional activation of the CT muscle on the opposite side during phonation caused an obviously noticeable rotation of the posterior glottis to the paralytic side. The rotation was the most obvious when activation was added to the contralateral CT muscle, which was in a state of no contraction. The results indicate that the degree of the glottic rotation depends on the degree of activation of the CT muscle on the opposite side.

In clinical cases, therefore, unilateral SLN paralysis should be most markedly manifested during laryngeal behaviors in which the unaffected CT muscle is strongly activated. The test task employed in this study is one of those behaviors in which the contralateral CT muscle is significantly activated. The purpose of low-pitched phonation was to have a control condition in which the CT muscle activity was low. The results of the present clinical study indicate that the test task consisting of low-pitched phonation followed by high-pitched phonation provides a good clinical examination for unilateral SLN paralysis. Its problems are that 1) some patients cannot perform the task, 2) the test does not manifest SLN paralysis when marked scar formation interferes with movements of the laryngeal framework, and 3) an incomplete SLN paralysis may not be manifested by the test. Nevertheless, this test is a relatively simple and noninvasive procedure, and it is not time-consuming. It is easily employed in daily laryngological practice.

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ACOUSTIC ANALYSIS OF VOCAL INSTABILITY IN CEREBELLAR DYSFUNCTIONS

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Acoustic analysis of vocal instability during the production of isolated vowels, including computation of mean fundamental frequency (F0), period-to-period variability (jitter), pitch fluctuations, and between-trial variation of F0, was performed in 20 patients with cerebellar cortical dysfunction. Eleven subjects suffered from purely cerebellar atrophy (CA). In the remaining 9 patients olivopontocerebellar atrophy (OPCA) had been diagnosed. A subgroup of both the CA and OPCA subjects presented with enlarged pitch fluctuations and/or increased jitter values. It is conceivable that asymmetrically distributed motor deficits at the laryngeal level and altered gain settings of laryngeal and/or respiratory reflexes account for the observed phonatory instability. Moreover, 5 of the 20 cerebellar patients had a pitch level exceeding the upper limit of the normal range. Presumably, this deviation reflects increased vocal effort.

KEY WORDS — acoustic analysis, ataxic dysarthria, cerebellar dysarthria, cerebellar dysphonia.

INTRODUCTION

Besides articulatory inaccuracy and scanning speech rhythm, dysfunctions of the cerebellum may give rise to phonatory disturbances.[1] According to the comprehensive auditory-perceptual studies of Darley et al[2,3] and Brown et al,[4] harsh voice quality and monopitch belong to the 10 most prominent cerebellar speech deficits. Moreover, Kluin et al[5] considered pitch fluctuations a salient feature of cerebellar dysarthria.

Increased period-to-period variability (jitter) of fundamental frequency (F0), presumably, represents the acoustic correlate of perceived harshness.[6] Audible pitch fluctuations, in contrast, rather reflect directional shifts of F0 over longer sequences of vocal fold oscillations ("long-term phonatory instability").[7] The reported findings, thus, indicate an impairment of both period-to-period and long-term phonatory stability concomitant with cerebellar syndromes. Accordingly, oscillographic analyses of word and sentence utterances from patients with cerebellar disease or lesions documented an "instability of frequency" within single vocalic segments.[8] By means of electroglottography, moreover, increased variability of laryngeal vibrations was found in ataxic subjects.[9]

Rather than connected speech, sustained vowels provide a feasible test to examine phonatory stability, avoiding, for example, interference from speech prosody and articulation.[6,10] So far, no parametric data on this task are available from patients with cerebellar disorders. Admittedly, irregular changes of the F0 contours of isolated vowels were found in patients suffering from Friedreich's ataxia.[11,12] This disease, however, represents a disorder of the spinocerebellar and bulbocerebellar tracts. The cerebellum proper shows no relevant involvement on neuroradiologic investigations.[13]

In order to clarify the impact of cerebellar dysfunction on phonatory stability during the production of sustained vowels, investigation of patients with cerebellar disorders is required.[14-16] Therefore, acoustic measurements, including computation of period-to-period variability (jitter), pitch fluctuations, and between-trial F0 variation, were performed in subjects with cerebellar ataxia. Since the disease process may extend beyond the cerebellum, the data obtained from subjects with disorders restricted to this organ and those from patients with additional extracerebellar deficits were pooled separately.

METHODS

Subjects. Twenty patients suffering from cerebellar ataxia participated in this study. Table 1 shows the relevant clinical data. Subjects more than 70 years old were excluded from the present investigation, since age-related degenerative changes of laryngeal tissue may compromise vocal stability as well.[17-19]

A diagnosis of purely cerebellar atrophy (CA) was assumed in 11 patients on the grounds of an ataxic syndrome concomitant with disease restricted to the cerebellum at cranial computed tomography (CCT)

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TABLE 1. CLINICAL DATA OF PATIENTS WITH CEREBELLAR ATROPHY

Subject	*Age (y)*	*Sex*	*Duration (y)*	*Heredity*	*Ataxia of Gait*	*Ataxia of Upper Limbs*	*Cranial Nerves*	*Tendon Reflexes (Legs)*	*Babinski Sign*	*Autonom/ Extrapyr*
Purely cerebellar atrophy										
CA1	36	M	1	+	1	–	n	n	–	–
CA2	61	F	2	–	1	1	n	n	–	–
CA3	56	M	3	–	1	1	n	n	–	–
CA4	54	M	4	–	2	1	n	n	–	–
CA5	59	M	4	–	2	2	n	n	–	–
CA6	68	F	5	–	1	–	n	n	–	–
CA7	64	F	13	+	3	2	n	n	–	–
CA8	54	M	14	+	2	1	n	n	–	–
CA9	55	M	17	–	2	2	n	n	–	–
CA10	58	M	<1	–	1	1	n	n	–	–
CA11	63	F	<1	–	3	2	n	n	–	–
Olivopontocerebellar atrophy										
OPCA1	44	M	3	–	2	2	n	n	–	+
OPCA2	55	M	4	+	2	2	n	n	–	+
OPCA3	60	M	5	–	2	2	III, IX, X	n	–	–
OPCA4	65	M	7	–	2	1	n	n	–	+
OPCA5	30	M	8	–	2	1	IX, X, XII	n	–	–
OPCA6	55	M	10	–	3	1	VI	exa	+	+
OPCA7	62	F	11	+	1	1	n	n	–	+
OPCA8	40	M	15	–	3	2	III, IV, VI	n	–	–
OPCA9	44	F	21	–	2	2	n	exa	+	–

Autonom — signs of autonomic dysfunction, Extrapyr — extrapyramidal signs, + — present, – — absent, 1 — slightly impaired, 2 — moderately impaired, 3 — severely impaired, n — normal, exa — exaggerated.

and/or magnetic resonance imaging (MRI). Two CA patients presented with ataxia of acute onset due to cerebellitis of unknown cause and lithium intoxication, respectively. In 9 CA subjects a slowly progressive development of the cerebellar deficits had occurred. Family histories indicated autosomal dominant cerebellar ataxia[20] in 3 of the latter patients. The disorder of the remaining chronic CA subjects was identified as idiopathic cerebellar ataxia.

Nine of the total of 20 patients showed, in addition to being ataxic, cranial nerve deficits, pyramidal disorders, extrapyramidal dysfunctions, and/or autonomic failure. It had to be assumed, therefore, that the disease extended beyond the cerebellum. Olivopontocerebellar atrophy (OPCA) was diagnosed in these subjects. Besides cerebellar atrophy, 7 of them showed an involvement of the brain stem on CCT and/or MRI scans. Since extracerebellar disorders such as cranial nerve deficits and extrapyramidal signs might influence phonatory stability, the data obtained from the patients with CA and OPCA were pooled separately.

The control group included 30 healthy subjects who had never suffered from diseases or traumata affecting the central nervous system or the cranial nerves (16 men and 14 women; age 26 to 67 years).

Test Material and Data Recording. The various vowel categories differ with respect to vocal tract configuration (eg, tongue position or lip rounding). In order to assess possible influences of orofacial posturing on phonatory stability, 4 different vowel categories were considered for analysis: /a/, /i/, /u/, and /y/. The subjects had to produce each of the 4 vowels 6 times in a randomized order, amounting to a total of 24 trials per individual. Since age-related changes in body physiology may compromise maximum performance tasks, the vowel productions were limited to a comfortable duration of 2 to 3 seconds.[21]

The investigation took place in a sound-treated booth with high-quality equipment (DAT recorder PCM 2000, Sony, Japan, or tape recorder Nagra IV-S, Nagra-Kudelski, Switzerland; condenser microphone C-48, Sony, Japan). After low-pass filtering (cutoff 9 kHz) and digitization (12-bit resolution, sampling frequency 20 kHz), the data were fed into an LSI 11/73 laboratory computer for off-line analysis.

Acoustic Parameters. By means of a pitch extraction algorithm the time course of F0 during vowel productions (F0 contour) was calculated.[22] The extracted F0 contours were shortened by 10% at both ends to exclude vocalic onset and offset from analysis.

TABLE 2. MEAN FUNDAMENTAL FREQUENCY VALUES OF CONTROL SUBJECTS AND CEREBELLAR PATIENTS

Subject	*Sex*	*Mean F0 (Hz)*
Purely cerebellar atrophy		
CA1	M	121
CA3	M	103
CA4	M	110
CA5	M	131
CA8	M	145
CA9	M	100
CA10	M	96
CA2	F	204
CA6	F	215
CA7	F	258
CA11	F	265
Olivopontocerebellar atrophy		
OPCA1	M	113
OPCA2	M	117
OPCA3	M	91
OPCA4	M	185
OPCA5	M	87
OPCA6	M	131
OPCA8	M	135
OPCA7	F	233
OPCA9	F	187

Normal range for men is 84 to 143 Hz; for women, 172 to 227 Hz.

In order to obtain a measure of F0 jitter, ie, the period-to-period variability, the F0 contour was low-pass-filtered: 3-point filter: $x'(i) = [x(i-1) + 2x(i) + x(i+1)]/4$.[23] This procedure resulted in the separation of 2 components: the smoothed F0 contour and a high-frequency residuum. Jitter then was calculated as the absolute mean error of the successive periods with respect to the smoothed F0 contour. This parameter can be considered an acoustic correlate of perceived vocal harshness.

In a further step of analysis, the regression line of each smoothed F0 contour was computed. The standard deviation of the glottal periods of the residual F0 contour, obtained after subtraction of the regression line, was considered to reflect long-term phonatory instability,[7] ie, F0 shifts extending over a sequence of vocal fold oscillations giving rise to audible pitch fluctuations.

For each subject the overall mean F0 across the 24 vowel productions was determined, representing the acoustic correlate of perceived pitch level.

Besides overall pitch level, the mean F0 of each single vowel production was calculated. The standard deviation of the measures obtained from each individual was considered the between-trial F0 variation.

In accordance with psychoacoustic standards, mean F0 and the 3 measures of variability were logarithmically transformed into semitones by using 50 Hz as a reference value. The parameters of variability then were expressed in percent of mean F0.

RESULTS

Overall Pitch Level and Between-Trial Pitch Variation. Individual overall mean F0 was determined across the 24 vowel productions obtained from each subject. Table 2 shows the values of the cerebellar patients as well as the normal range. The pitch levels of the control speakers accord well with data reported in the literature.[24] Five ataxic patients (CA 7, 8, and 11; OPCA 4 and 7), 2 men and 3 women, had an overall mean F0 above the normal range (Table 2). However, in 2 instances the increase amounted to a few hertz only. None of the cerebellar subjects showed consistent pitch lowering.

Besides overall pitch level, mean F0 across the 6 productions of each of the 4 vowel categories was determined. Analysis of variance revealed a significant vowel effect ($p < .01$) due to a lowered mean F0 during production of /a/ as compared to the 3 high vowels. This difference was found in all 3 subject groups considered, ie, control speakers and CA and OPCA patients.

Because of the systematic F0 differences between /a/, on the one hand, and /i/, /y/, and /u/, on the other, it seemed feasible to restrict computation of between-trial pitch variation to the high vowels. Thus, a total of 18 trials was considered in each subject. Only 1 patient (OPCA8) had a value exceeding the normal range (Fig 1).

Period-to-Period and Long-term Vocal Instability. Two-way analyses of variance were performed for the parameters "vocal jitter" and "long-term F0 fluctuations." Since male and female speakers differ in mean pitch level (see Table 2), systematic differences with respect to measures of vocal instability are to be expected. To account for the unbalanced proportion of the sexes within the cerebellar group, statistical computations were restricted to male subjects. Table 3 presents the mean values of both variables for the 3 subject groups and the 4 vowel categories. With respect to both parameters, significant group and vowel effects were disclosed ($p < .01$ in all instances). The groups of CA and OPCA subjects had higher jitter and more pronounced long-term fluctuations during production of /a/. With respect to vocal jitter, moreover, a significant interaction between the factors "group" and "vowel" was detected due to a particularly strong vowel effect in

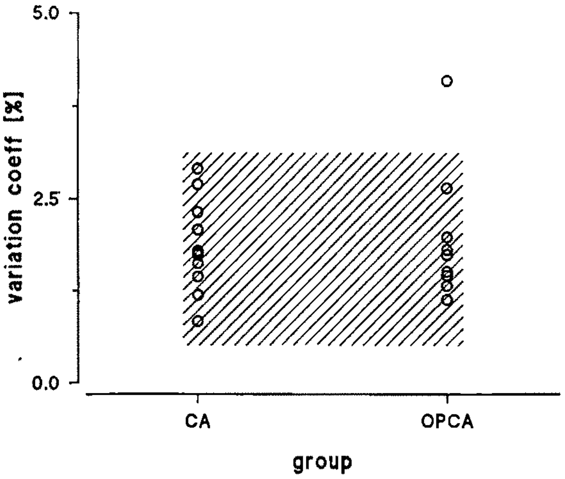

Fig 1. Intraindividual variation of fundamental frequency (F0) across 18 trials of sustained high vowels (6 productions each of /i/, /u/, and /y/) in 11 subjects with purely cerebellar atrophy (CA) and 9 patients suffering from olivopontocerebellar atrophy (OPCA). Ordinate presents standard deviation in percent of individual mean F0 (logarithmically expressed in semitones). Hatched area represents normal range (male and female subjects pooled).

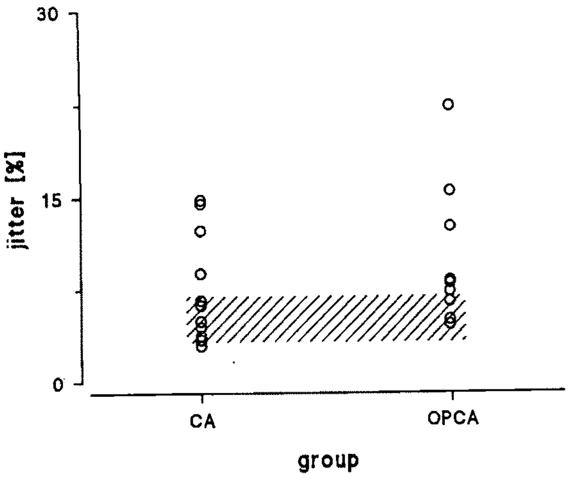

Fig 2. Glottal period-to-period variability (vocal jitter) in CA and OPCA subjects computed across 18 trials of sustained high vowels (6 productions each of /i/, /u/, and /y/). Ordinate provides jitter in percent of individual mean F0 (logarithmically expressed in semitones). For further explanation, see Fig 1.

the OPCA group. The vowel effects and group by vowel interactions could be traced to /a/, indicating that the 3 high vowels yielded comparable results. In order to describe the group differences more closely, the data for /i/, /y/, and /u/ were pooled and /a/ was discarded from further analysis. In the following sections, thus, the individual variability measures represent median values across a total of 18 productions each.

Figure 2 shows the distribution of the computed individual medians of F0 jitter within both patient groups. Four of the 11 CA subjects (Fig 2, from the top: CA 10, 5, 11, and 2) and 6 of the 9 OPCA patients (Fig 2, from the top: OPCA 6, 8, 1, 7, 3, and 4) had a jitter value above the normal range. An example of increased period-to-period variability is shown in Fig 3. Only 1 subject (CA9) presented with reduced jitter.

Increased long-term phonatory instability, measured from the smoothed F0 contours after correction for linear trends, could be detected in 6 CA subjects (Fig 4, from the top: CA 7, 11, 10, 2, 5, and 3) and in 5 OPCA subjects (Fig 4, from the top: OPCA 6, 8, 9, 5, and 3). Apart from subject CA7, the F0 contours of the patients with long-term phonatory instability included irregularly distributed shifts of F0 that were perceived as pitch fluctuations (Fig 3). The CA patient with the most pronounced pitch fluctuations (CA7) showed quasi-rhythmic modulation of the F0 contour at a frequency of 2.8 Hz.[25] Perceptual evaluation yielded audible voice tremor. Intermittent rhythmic modulation of the F0 contour concomitant with perceived voice quivering could be noted in patient CA11 as well (Fig 3). All CA subjects with increased jitter also presented with pitch fluctuations; the OPCA group showed only a partial overlap in this regard.

TABLE 3. MEAN VOCAL JITTER AND PITCH FLUCTUATIONS AS PERCENTAGE OF INDIVIDUAL MEAN FUNDAMENTAL FREQUENCY

		/i/	/y/	/u/	/a/
Controls (N = 16)	Jitter	7	6	5	7
	Fluctuations	20	21	21	21
CA subjects (N = 7)	Jitter	7	7	10	12
	Fluctuations	24	23	23	28
OPCA subjects (N = 7)	Jitter	13	11	10	20
	Fluctuations	24	22	24	32

Means are based on 6 trials per subject. All subjects are men.

CA — purely cerebellar atrophy, OPCA — olivopontocerebellar atrophy.

Comparison of Clinical and Acoustic Data. The patients with impaired phonatory stability did not represent a homogeneous sample with respect to clinical data such as degree of articulatory deficits or severity of nonspeech ataxic signs (Table 1). This holds true for the CA as well as the OPCA subjects. For example, patient CA2 had increased jitter and pitch fluctuations in the presence of slight ataxia and discrete articulatory disorders. In contrast, subject CA9 was unimpaired with respect to vocal stability despite more pronounced disturbances of articulation, upper limb coordination, and stance regulation.

DISCUSSION

A subgroup of the patients with cerebellar ataxia presented with reduced stability of vocal fold oscillations, in terms of increased jitter and pitch fluctua-

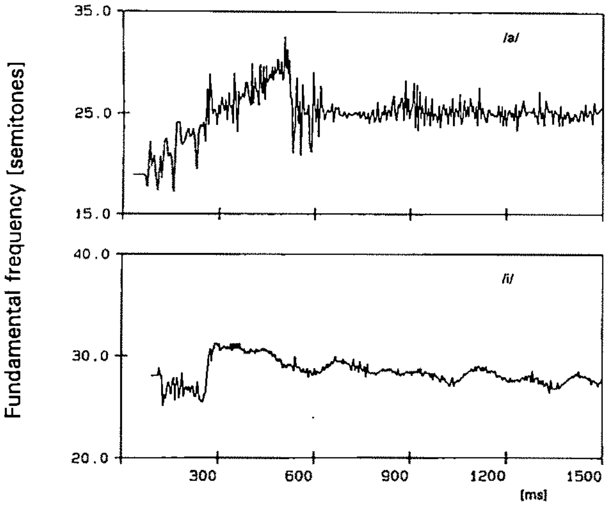

Fig 3. Two examples of sustained vowel production obtained from subject CA11. Unfiltered F0 contours show irregular (upper and lower panel) and rhythmic (lower panel) pitch fluctuations, as well as increased jitter (upper panel).

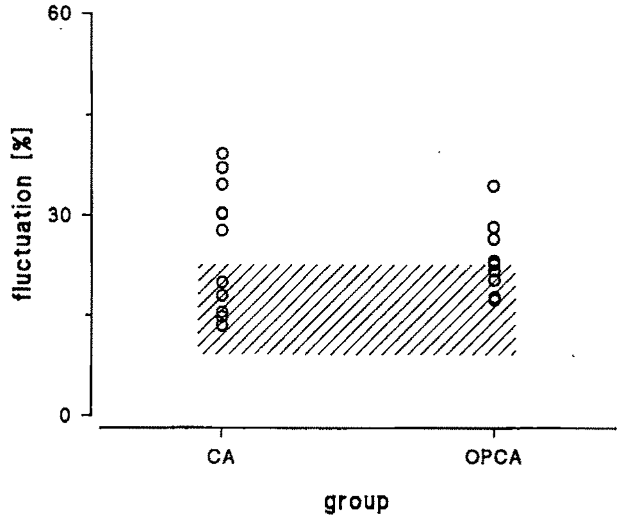

Fig 4. Pitch fluctuations (long-term phonatory instability) in CA and OPCA patients calculated across 18 trials of sustained high vowels (6 productions each of /i/, /u/, and /y/). Ordinate presents standard deviation of smoothed F0 contour after correction for linear trends in percent of individual mean F0 (logarithmically expressed in semitones). Two CA subjects had nearly similar values (fourth circle from top). For further explanation, see Fig 1.

tions, during sustained phonation. This holds true both for subjects with disorders restricted to the cerebellum, as well as for OPCA patients. It must be assumed, therefore, that cerebellar dysfunction on its own can lead to impaired phonatory functions.

Since OPCA involves extracerebellar structures as well, these patients may, in addition to ataxia, present with bulbar deficits, upper motor neuron signs, and/or parkinsonian-like symptoms.[20,26,27] Darley et al[2,3] and Brown et al[4] found harsh voice quality to be among the 10 most prominent features of bulbar, suprabulbar, and parkinsonian dysarthrias. Conceivably, then, extracerebellar disorders contributed to the increased jitter values of the OPCA subjects. In contrast, Darley et al and Brown et al did not observe pitch fluctuations in syndromes of the upper and lower motor neurons, nor in Parkinson's disease. Presumably, then, extracerebellar dysfunctions are not relevant for long-term phonatory instability.

The rate of vocal fold oscillations mainly depends on the folds' tension or stiffness, on the subglottal air pressure, and on the mass of the vibrating structures.[28] Maintenance of a fairly constant F0 during sustained vowel production thus requires ongoing control of the activity of laryngeal and respiratory muscles. Both exteroceptive reflexes, mainly originating from laryngeal joint receptors, and proprioceptive mechanisms seem to contribute to the monitoring of laryngeal muscle contraction during sustained phonation.[29-31] Moreover, regulation of intercostal and abdominal muscles during speech breathing might rely on proprioceptive activity as well.[28,32] Since the cerebellum seems to function as a control system adjusting the gain of proprioceptive loops mediated by extracerebellar structures,[33] it is quite conceivable that cerebellar disorders give rise to pitch fluctuations.

A subgroup of both the CA and OPCA patients had increased F0 jitter. Period-to-period variability of F0, representing short-term changes within a range of a few milliseconds, cannot be accounted for by impaired reflex monitoring of phonatory settings. For example, biomechanical differences between the two vocal folds have been suggested to cause increased F0 jitter.[6] Thus, asymmetrically distributed hypotonia at the laryngeal level might give rise to differences between the two vocal folds in overall tension or in vibrating mass and, as a consequence, lead to irregular vocal fold oscillations. In accordance with these assumptions, laryngoscopic investigations disclosed abnormal tone of the vocal folds and irregular adduction gestures, respectively, in ataxic subjects.[9,34,35] Alternatively, jitter might reflect small and rapid variations of the tension of both vocal folds due to the inherent firing irregularities within a finite pool of motoneurons.[6] In consideration of this model, decreased activity of the motoneuron pool could give rise to more pronounced "ripples" of the overall tension curve. However, this explanation is inconsistent with the fact that the patients did not present with a lower mean F0, as one would expect in the case of reduced vocal fold tension.

The patients with phonatory instability in terms of increased jitter or pitch fluctuations were not homogeneous with respect to clinical data such as degree of articulatory deficits, severity of nonspeech ataxic findings, and bulbar signs. Since the cerebellar cortex is characterized by functional compartmentalization,[36] the discrepancies of speech motor symptoms might simply reflect a nonhomogeneous distribution of cerebellar disease.

With respect to mean F0, a significant between-vowel difference was disclosed for all 3 subject groups in that production of /a/ gave rise to a consistently lower pitch than /i/, /y/, or /u/. This intrinsic pitch effect of the high vowels is often ascribed to biomechanical lingual-laryngeal coupling in terms of a tilting and stretching of the vocal folds consequent upon tongue raising. Between-vowel differences of vocal jitter and pitch fluctuations were found in the 2 patient groups only. Assuming lingual-laryngeal coupling, the larynx should be less constrained by the tongue in articulation of /a/. The relatively more pronounced degrees of freedom under these conditions may give rise to reduced vocal stability, ie, increased jitter and fluctuation values. The normal speakers presented with similar F0 stability under all vowel conditions, and thus seem to be less sensitive to reduced lingual-laryngeal coupling.

A sporadically increased overall pitch level of cerebellar patients has been reported in the literature.[1] Five ataxic patients of the present study showed an overall mean F0 above the normal range. It is known that altered sensory feedback, eg, anesthesia of laryngeal structures, results in a higher average F0 during connected speech.[37] This effect has been interpreted as reflecting enlarged vocal effort to overcome these disturbances. Conceivably, disturbed regulation of the tension of the internal laryngeal muscles gives rise to similar strategies of compensation in a subgroup of patients.

Despite pitch fluctuations, the between-trial variability of F0 was within the normal range in nearly all patients. It must be assumed, therefore, that a resetting of the F0 shifts occurred. Thus, regulatory mechanisms of phonatory stability apart from proprioceptive loops should still be intact. Conceivably, auditory monitoring subserves the resetting of F0 shifts as seen in the examples in Fig 3.

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ANATOMIC CONSIDERATIONS IN COMPLICATIONS OF ENDOSCOPIC AND INTRANASAL SINUS SURGERY

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Complications of endoscopic and classic intranasal sinus surgery have been documented by case reports and large retrospective reviews. The most serious complications have involved violation of the intracranial space or orbit. Hemorrhage has also proven to be a significant cause of morbidity, either of itself, or by limiting visualization and thus contributing to other injuries. Although numerous studies have been performed to determine various anatomic relationships, relatively few have addressed relationships that use practical reference points easily accessible to the endoscopic or intranasal sinus surgeon. In an effort to reduce the risk of complications such as blindness, orbital hematoma, and injury to the lacrimal sac or central nervous system, we have performed 50 cadaver dissections and measured 7 anatomic landmarks that may aid the surgeon in preventing these complications. We have also included a review of the literature concerning the complications of endoscopic and intranasal sinus surgery addressed herein.

KEY WORDS — complications, endoscopic sinus surgery.

INTRODUCTION

Intranasal and, more recently, endoscopic techniques have become popular approaches for the diagnosis and treatment of paranasal sinus disease. Distinct advantages of these approaches include the elimination of external scars and reduced patient pain and discomfort. A major limitation to either of these techniques, however, is the potential for the surgeon to become spatially disoriented and thereby increase the risk of serious complications. Complications have been reported to occur in up to 28.9% of endoscopic sinus surgery cases and 5% of traditional intranasal ethmoidectomies.[1-3] Although the majority of reported complications are less serious, those that involve violation of the orbit or central nervous system may be devastating.

To better understand the pertinent anatomy, Lucente and Schoenfeld[4] studied cadavers to document the distance from the pyriform aperture and angle of inclination from the floor of the nasal cavity to various intranasal structures. Calhoun et al[5] also used cadavers to determine linear and angular measurements of lateral nasal wall landmarks between each other and the nasal sill, while Rontal and Rontal[6] studied whole mount sections of the paranasal sinuses to determine qualitative relationships between anatomy and endoscopic surgery complications. Although these studies have provided useful information, the locations of certain extremely important anatomic landmarks, such as the ethmoidal arteries, which aid in identifying the skull base and may contribute to hemorrhage and orbital hematomas, and the optic nerve, which may be injured during the surgery, have yet to be defined employing surgically convenient points of reference. In this anatomic study, cadavers were used to quantify linear and angular relationships of various structures pertinent to surgical complications such as blindness, orbital hematoma, and injury to the lacrimal sac or central nervous system. The anatomic data presented here are intended to aid the intranasal or endoscopic sinus surgeon in the prevention of these untoward sequelae.

MATERIALS AND METHODS

Fifty embalmed half-heads from 50 different cadavers, 48 left-sided and 2 right-sided, were used for a total of 50 sets of measurements. The cohort consisted of 24 white men, 25 white women, and 1 black woman. As the half-heads had been separated from their respective cadavers prior to the study, the ages of the subjects at death were unavailable, but were estimated to be between the ages of 40 and 90 years. None of the half-heads demonstrated anatomic anomalies or evidence of trauma or prior surgery.

Angular measurements were made to the nearest degree by placing the origin of a transparent protractor at the base of the columella, and aligning the 0° angle with the floor of the nasal cavity. A ruler was placed through the naris and linear measurements were made to the nearest millimeter from the base of

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TABLE 1. EXPERIMENTAL RESULTS

	LS		*AU*	*PU*	*BL*	*AEA*		*PEA*		*ON*	
	A	*d*	*d*	*d*	*d*	*A*	*d*	*A*	*d*	*A*	*d*
Total (n = 50)											
Mean	50	44	47	49	58	51	64	42	70	34	83
SD	5	5	5	5	5	5	5	5	5	3	7
Range	35-65	30-55	32-60	37-58	47-68	40-62	56-75	33-58	60-82	28-40	65-95
Male (n = 24)											
Mean	49	45	48	50	59	51	67	41	73	34	85
SD	7	5	6	6	5	5	4	4	5	3	7
Range	35-60	30-55	32-60	37-58	48-68	40-62	60-75	33-51	63-82	28-39	70-95
Female (n = 26)											
Mean	51	44	46	48	56	51	62	43	68	34	80
SD	7	4	4	5	4	5	4	5	5	3	6
Range	38-65	37-51	35-52	37-56	47-64	41-60	56-73	33-58	60-78	30-40	65-94

A — angle of inclination (in degrees), d — distance (in millimeters), LS — lacrimal sac, AU — anterior uncinate process, PU — posterior uncinate process, BL — basal lamella, AEA — anterior ethmoid artery, PEA — posterior ethmoid artery, ON — optic nerve.

the columella. Distance and angle of inclination, or distance only, were recorded for the various structures.

The inferior, anterior, and posterior attachments of the nasal septum were divided with a scalpel, allowing the septum to be reflected superiorly at the skull base. The middle and superior turbinates were then reflected superiorly en bloc at the skull base in like fashion, by means of an additional parasagittal incision from the middle meatus to the skull base. The anterior and posterior limits of the uncinate process were identified and distances measured. The basal lamella was identified and distance recorded at an angle 40° from the floor of the nasal cavity, approximating the approach of the surgeon. The anterior ethmoid air cells, posterior ethmoid air cells, and skull base mucosa were then removed with forceps. Angles of inclination and distances were measured for the anterior and posterior ethmoid arteries. The intersinus septum of the sphenoid sinus was removed when present, and the mucosa stripped from the sinus with forceps. The most anterior portion of the optic nerve immediately adjacent to the paranasal sinuses was then noted. After dissection of the optic nerve, the shortest distance to the columella and angle of inclination were recorded. A Lynch incision was then made and the lacrimal sac and fossa were identified. A probe was used to puncture the lacrimal fossa and enter the nasal cavity, and distance and angle to the probe were recorded.

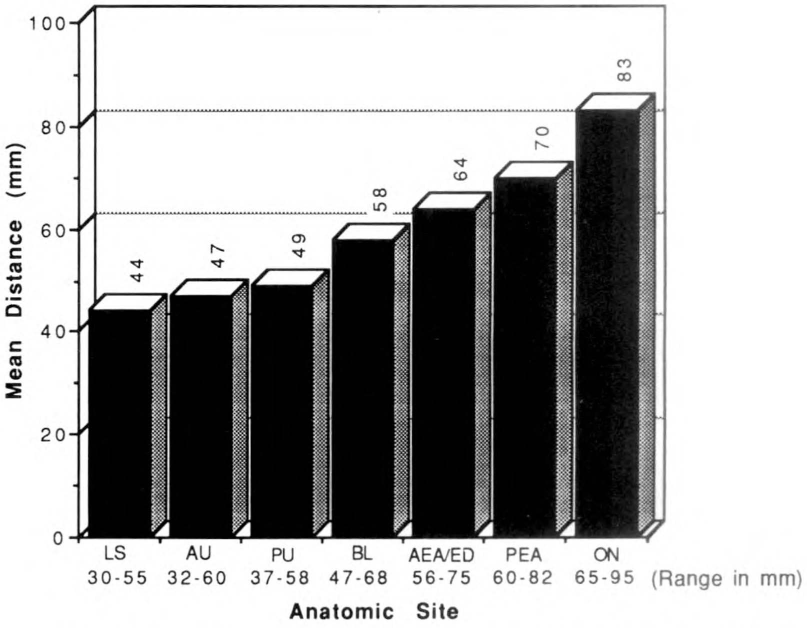
RESULTS

The mean, standard deviation, and range of each measurement are shown in Table 1 for the entire cohort, as well as for the male and female populations. The mean distances are also depicted graphically in the Figure. Distances for the anterior ethmoid artery–ethmoid dome, posterior ethmoid artery, optic nerve, and basal lamella were significant between the male and female subjects at p values of .0001, .0001, .0060, and .0344, respectively. No comparisons between black and white cadavers or left and right half-heads were possible because of an insufficient number of nonwhite and right-sided specimens, but previous studies reveal no significant differences between races or sides of the head.[4,5]

DISCUSSION

Complications resulting from endoscopic or intranasal sinus surgery have been reported to occur in 5% of intranasal and approximately 15% to 29% of endoscopic sinus procedures (Table 2[1-3]). Although not all of these complications are serious, those that are serious are potentially catastrophic. Generally, the more serious injuries involve violation of the superior or lateral landmarks, resulting in intracranial or orbital complications, and occur when the surgeon becomes disoriented. The risk of disorientation may be compounded by bleeding, resulting in further obscuring of anatomic landmarks, and by the fact that many intranasal procedures are performed without the benefit of binocular vision and depth perception.

Major hemorrhage, occurring in 2.1% to 5.6% of cases, has been identified as the most common serious complication of intranasal and endoscopic sinus surgery.[1-3] While a review of the literature does not indict the ethmoidal arteries as the major cause, clearly these vessels are potential sources of bleeding and represent important surgical landmarks indicating the level of the skull base. Additionally, when injured, the ethmoid arteries may retract into their bony canals and bleed into the orbit, thus causing an



Graphic representation of mean distances, measured from base of columella, for each structure. Ranges for each measurement are noted on x-axis beneath anatomic abbreviation. LS — lacrimal sac, AU — anterior uncinate process, PU — posterior uncinate process, BL — basal lamella, AEA/ED — anterior ethmoid artery–ethmoid dome, PEA — posterior ethmoid artery, ON — optic nerve.

orbital hematoma, the most common ophthalmic complication of intranasal and endoscopic surgery.[1-3] Although orbital hemorrhage occurred in only 0.7% of the intranasal ethmoidectomies reported by Freedman and Kern[3] and 2.8% to 5.6% of endoscopic ethmoidectomies reported by Stankiewicz,[1,2] these complications can result in blindness. Loss of visual acuity due to an orbital (retrobulbar) hematoma is thought to be a result of decreased ocular perfusion and resultant ischemia of the eye. As orbital pressure approaches or exceeds venous pressure, the resultant venous congestion decreases central retinal arterial flow, thus causing retinal ischemia.[7] With further bleeding into the bony confines of the orbit, the expanding hematoma may eventually approach or exceed the systolic blood pressure, further compromising the central retinal artery flow.[8,9] A review of the US literature since 1966 reveals 30 reported cases of orbital hematoma associated with endoscopic or intranasal sinus surgery, with 13 of these resulting in permanent blindness. Twelve of these cases resulted in permanent uniocular blindness, 1 case resulted in permanent bilateral blindness, 1 case resulted in temporary uniocular blindness, and 16 cases were treated without complication.[1,7,8,10-14] Causes of the hematomas included 1 reported injury of the anterior ethmoidal artery, 1 hematoma associated with posterior ethmoidal artery bleeding, and 1 case involving bleeding from both ethmoidal areas. In the remaining 27 cases the site of injury was not specified. The procedures in which these complications occurred included 21 intranasal ethmoidectomies, 1 septoplasty-polypectomy, and 8 endoscopic ethmoidectomies.[1,7,8,10-14]

Forty-two intracranial complications from endoscopic or intranasal sinus surgery were identified in the US literature since 1966. Twenty-three of these cases were associated with intranasal procedures and

TABLE 2. REPORTED COMPLICATIONS OF INTRANASAL AND ENDOSCOPIC ETHMOIDECTOMY

	Freedman and Kern[3] (n = 565)		*Stankiewicz 1987[1] (n = 90)*		*Stankiewicz 1989[2] (n = 180)*	
Complication	*No.*	*%*	*No.*	*%*	*No.*	*%*
Overall	28	5.0	26	28.9	28	15.6
Total hemorrhage	12	2.1	5	5.6	5	2.8
Intraoperative	4	0.7	2	2.2	2	1.1
Postoperative	8	1.4	3	3.3	3	1.7
Orbital hematoma	4	0.7	5	5.6	5	2.8
Cerebrospinal fluid leak	1	0.2	1	1.1	2	1.1
Temporary blindness	0	0	1	1.1	1	0.6
Permanent blindness	0	0	0	0	0	0
Central nervous system injury	0	0	0	0	0	0
Lacrimal sac injury	0	0	0	0	0	0
Death	0	0	0	0	0	0

19 cases were associated with endoscopic procedures. The most common complication resulting from intracranial penetration has been cerebrospinal fluid leak, with 34 cases reported.[1-3,11,13-20] The site of injury was identified in 27 of these cases, with 13 involving the cribriform plate, 9 involving the ethmoid roof, and 5 involving the sphenoid. Additionally, 4 deaths attributed to anterior cranial fossa hemorrhage in association with endoscopic or intranasal sinus procedures have been reported.[11,13,18] One of the deaths involved a suction tip that pierced the ethmoid dome into the anterior cranial fossa to a depth of greater than 2 cm as confirmed by intraoperative radiography.[11,13] Maniglia[18] also has reported a nonfatal case in which an endoscope had penetrated 7 to 8 cm deep to the cribriform plate.

The results of our data would suggest that whenever a surgical instrument is introduced into the nose for a distance greater than 5.5 cm, the risk of encountering the ethmoid arteries is increased. Additionally, when the angle of the instrument exceeds 40°, respective to the floor of the nasal cavity, and the instrument is more than 5.5 cm into the nose, the risk of violation of the skull base increases.

Direct injury to the optic nerve and resultant blindness is a rarely reported complication of intranasal surgery. It is the authors' suspicion, however, that many of these cases appear in court but never show up in the medical literature. Trauma to this nerve is facilitated by its relatively fixed position as it enters the bony optic canal and its close proximity to the posterior ethmoid and sphenoid sinuses. Additionally, congenital or disease-induced dehiscence of protective bone and ethmoidal air cells that expand to partially surround the nerve may further contribute to the potential of injury of this structure. Our search of the US literature was able to identify only 7 cases of visual defects attributed to optic nerve injury in association with endoscopic or intranasal sinus surgery. These involved 2 cases of bilateral blindness following bilateral endoscopic ethmoidectomies, 1 case of bilateral blindness that occurred during removal of an osteoma of the sphenoid sinus, 1 case of unilateral blindness following bilateral endoscopic sinus surgery, 1 case of progressive blindness that occurred when the right optic nerve was exposed during an external and intranasal ethmoidectomy and sphenoidectomy, 1 case of mildly reduced visual acuity, and 1 case of a persistent visual field defect.[7,8,10,11,13,18]

Although the mean distance to the optic nerve in this study was 8.3 cm, our results would indicate the optic nerve may be encountered whenever an instrument is introduced 6.5 cm or more into the nose. The importance of this measurement becomes more apparent when one realizes that this is only 7 mm posterior to the point at which one would expect to encounter the basal lamella.

Clearly, close inspection of the preoperative computed axial tomography scans, with particular attention to the course of the optic nerve, can prove immensely valuable when surgery in this area is anticipated.

Although injury to the nasolacrimal system is less severe than the other complications discussed thus far, clearly this system is susceptible to injury in enlarging the maxillary sinus ostium. As the maxillary sinus ostium is enlarged anteriorly, the bony canal of the nasolacrimal duct is encountered. Since the bone in this area is much thicker, this often alerts the surgeon and may prevent further disruption of the membranous nasolacrimal duct. However, the bone protecting the nasolacrimal sac is much thinner and more easily violated. If the membranous nasolacrimal sac is violated during the procedure, drainage may be reestablished by spontaneous fistulization into the middle meatus of the nose. This may or may not be associated with stenosis and mild secondary epiphora. However, complete disruption of the nasolacrimal sac can result in total occlusion of the nasolacrimal system.

The results of this study would suggest that the potential for injury of the lacrimal sac could be quite high during any operation in the region of the superior aspect of the uncinate process. At this level, the lacrimal sac and fossa lie only 2 to 3 mm anterior to the free edge of the uncinate process.

Also included in this study were 2 anatomic landmarks that the endoscopic and intranasal sinus surgeon frequently finds useful in maintaining his or her orientation: the uncinate process and the basal lamella. The average distance from the columella to the anterior limit of the uncinate process has previously been reported to be 47.9 mm (male 48.7 mm, female 45.6 mm), which compares favorably to the value we obtained.[21] The posterior free edge of the uncinate process was found to have a mean depth of 49 mm (male 50 mm, female 48 mm), but no other study has documented this measurement for comparison. Since the uncinate process is an arc-shaped structure with its apex nearest to the columella, the measurements of its limits are almost identical.

The basal lamella, an important landmark separating the anterior and posterior ethmoidal air cells, varied from 47 to 68 mm, with a mean distance of 58 mm, as measured from the base of the columella. Although always located anterior to the point at which the optic nerve measurements were obtained, the basal lamella does not serve as a good landmark in preventing injury to the optic nerve. Indeed, the optic nerve can be located anterior to the point at which one might expect to encounter the basal lamella. Additionally, the distance between the optic nerve and the basal lamella varied from as little as 10 mm to over 41 mm.

CONCLUSION

In order to perform intranasal and endoscopic sinus procedures relatively safely, it is incumbent on the surgeon to think three-dimensionally, maintain points of reference, and become knowledgeable in the anatomy involving this region. To this end, the authors strongly advocate the placement of calibration marks on endoscopes and dissection instruments when feasible. This, in conjunction with an improved anatomic understanding and the information provided in this study, may assist the surgeon in maintaining the proper surgical orientation and reduce complications.

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MANAGEMENT OF THE TINNITUS PATIENT

Management of the Tinnitus Patient will be held in Iowa City, Iowa, October 14-15, 1994. For information, contact Regina Tisor, Workshop Secretariat; telephone (319) 356-2471; fax (319) 353-6739.

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RETROGRADE DILATION OF POSTSACCAL LACRIMAL STENOSIS

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The causes of nasolacrimal duct stenosis in adults can vary greatly. In general, the symptoms can also vary, but most cases share a tendency toward recurring inflammations in the prestenotic area. The treatment of these disorders is limited to either conservative therapy to control inflammation or surgically invasive measures. By using balloon catheters, usually applied in percutaneous transluminal coronary angioplasty (PTCA), dilation of the relative postsaccal stenosis can be performed under radiographic control. An exact diagnosis using various testing methods, including digital dacryocystography for detailed localization and documentation of any pathologic changes, is decisive to success. Only in cases of incomplete postsaccal stenosis is retrograde balloon dilation of the distal nasolacrimal duct indicated. A guide wire, designed for the PTCA balloon catheter set, is introduced via the canaliculus to the nasal cavity antegradely and caught with a thin hook and pulled from the naris, under visual control with an image converter. The balloon catheter is retrogradely threaded over the guide wire. The balloon is then placed at the site of the pathologic stenosis under radiographic control and dilated with high pressure. To ensure the permeability of the system, monocanalicular silicone intubation has to be performed immediately afterwards. This procedure has been performed successfully on 6 patients with a follow-up of 6 to 27 months. These initial results give rise to the hope that this minimally invasive, interdisciplinary technique represents a new alternative in the treatment of incomplete postsaccal lacrimal stenosis.

KEY WORDS — balloon catheter, postsaccal lacrimal stenosis, retrograde dilation, silicone intubation.

INTRODUCTION

Stenoses in the area of the nasolacrimal duct in adults can have various underlying causes. Most often they appear to be postinflammatory.[1] The ensuing epiphora is subjectively seen in most of these patients as bothersome. Additionally, the favorability for further inflammation in the prestenotic area is greatly increased. Untreated, this vicious cycle can result in grave consequences, including, in very extreme cases, orbital phlegmon. Early and adequate treatment of this disorder is therefore required. To date, treatment has been limited to conservative therapy to control inflammation and surgically invasive measures.

By employing the balloon catheter, primarily used in percutaneous transluminal coronary angioplasty (PTCA), a new, minimally invasive method was found as an alternative to the normally practiced surgical measures. (These standard procedures are performed either externally or endonasally.[2-6]) The obligatory prerequisites for this procedure are an exact diagnosis and use of radiography throughout

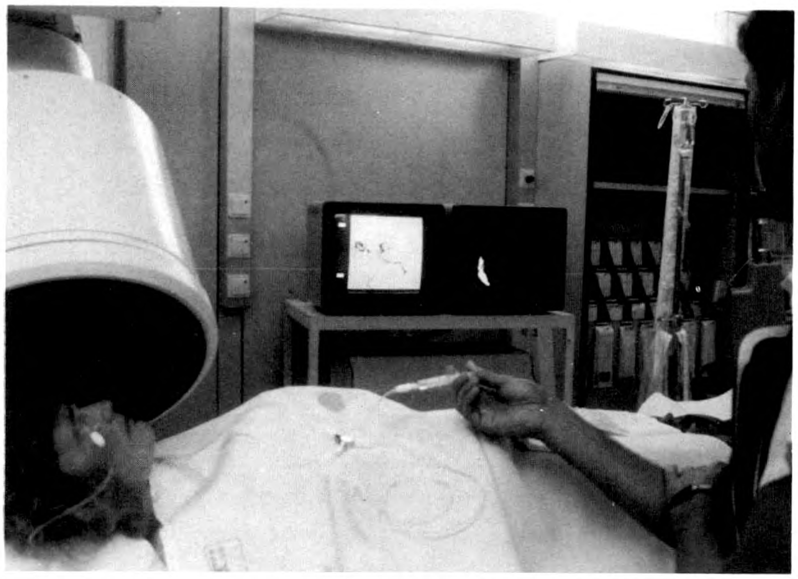

Fig 1. Digital dacryocystography in 31-year-old woman with bilateral incomplete postsaccal lacrimal stenosis.

From the Second Eye Clinic (Steinkogler, Huber, Kuchar) and the Clinic of Radiology (Karnel), University of Vienna, Vienna, Austria.
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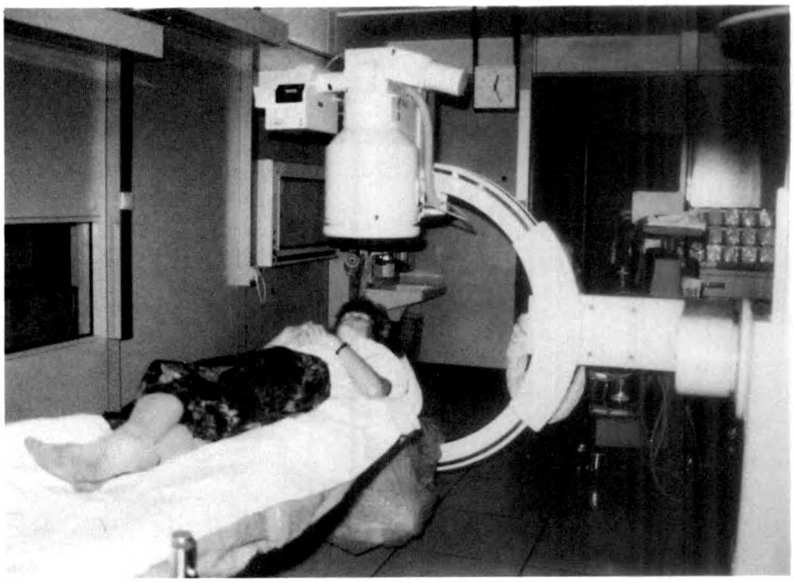

Fig 2. Computer-controlled x-ray unit with C-arc coupled to image-intensifying video system.

the procedure in order to ensure controlled manipulations.

MATERIAL AND METHODS

The preoperative diagnosis in patients with suspected stenosis of the nasolacrimal duct necessitates a series of tests and examinations, including a clinical examination of the lid apparatus, the lacrimal punctum excursion test, a dye test, a diagnostic irrigation of the lacrimal pathways, and finally, digital dacryocystography.[7,8] If an exact diagnosis is made and the patient's consent is given, a retrograde balloon dilation can directly follow dacryocystography (Fig 1). We use computerized x-ray equipment with a C-arc[8] (Fig 2) and a balloon catheter set like that used for PTCA (Monorail Speedy). The guide wire diameter is 0.14 mm. The balloon is 30.0 mm long and can be extended to a maximum diameter of 3.0 mm.

The plastic cannula of a Venflon 22-gauge catheter is placed in the superior canaliculi, and Xylocaine gel is instilled to anesthetize the mucous membrane in the lacrimal sac. Then the guide wire with its very soft and flexible tip is introduced through the plastic cannula in the lacrimal sac and directed toward the nasolacrimal duct and onward into the nasal cavity, under constant angiographic observation (Fig 3A). Since the soft tip usually wanders posteriorly (Fig 3B,C), it must be carefully pulled through the naris with a thin hook after topical anesthesia with Xylocaine spray. The soft balloon catheter is retrogradely (ie, cranially) threaded over the nasal end of the guide wire (which is coming out from the nasal cavity) and is carefully advanced up the nose and the lacrimal system. Again under radiographic observation, the balloon is exactly placed at the site of the stenosis (Fig 4A). The balloon is expanded by being filled with 0.3 to 0.4 mL of contrast medium 3 times, each time lasting 10 to 15 seconds (Fig 4B). Radiographic control allows an exact, controlled dilation procedure. Subsequently, a monocanalicular silicone intubation (Monoka) is performed through the superior lacrimal canaliculus to provide a shunt for the dilated system. It is left in place for approximately 3 months.

RESULTS

The retrograde, postsaccal balloon dilation has been performed to date on 4 women and 2 men ranging in age between 25 and 59 years. In 3 further patients, dilation was attempted, but it was not possible to insert the guide wire into the nasal cavity. The reason for the failure in these 3 cases was additional relative intrasaccal stenosis that was visible on digital dacryocystography. Intrasaccal changes represent an insurmountable obstacle when one is advancing the guide wire, since a noticeable change of direction must be made with the guide wire when passing from the canaliculi to the lacrimal sac. Therefore, a smooth and intact lacrimal sac mucous membrane surface is another prerequisite for this procedure, so that the guide wire tip glides forward smoothly. These 3 patients had to be excluded from the study. The dilation procedure was well tolerated in all 6 patients receiving topical anesthesia. Subjectively, the patients experienced merely the balloon dilation itself as uncomfortable. All patients received detumescent eyedrops postoperatively. Additional, antibiotic eyedrops were administered prophylactically in the first postoperative week.

In none of the patients did acute inflammation occur after dilation. During the postoperative observation period (see Table) of 6 to 27 months (mean

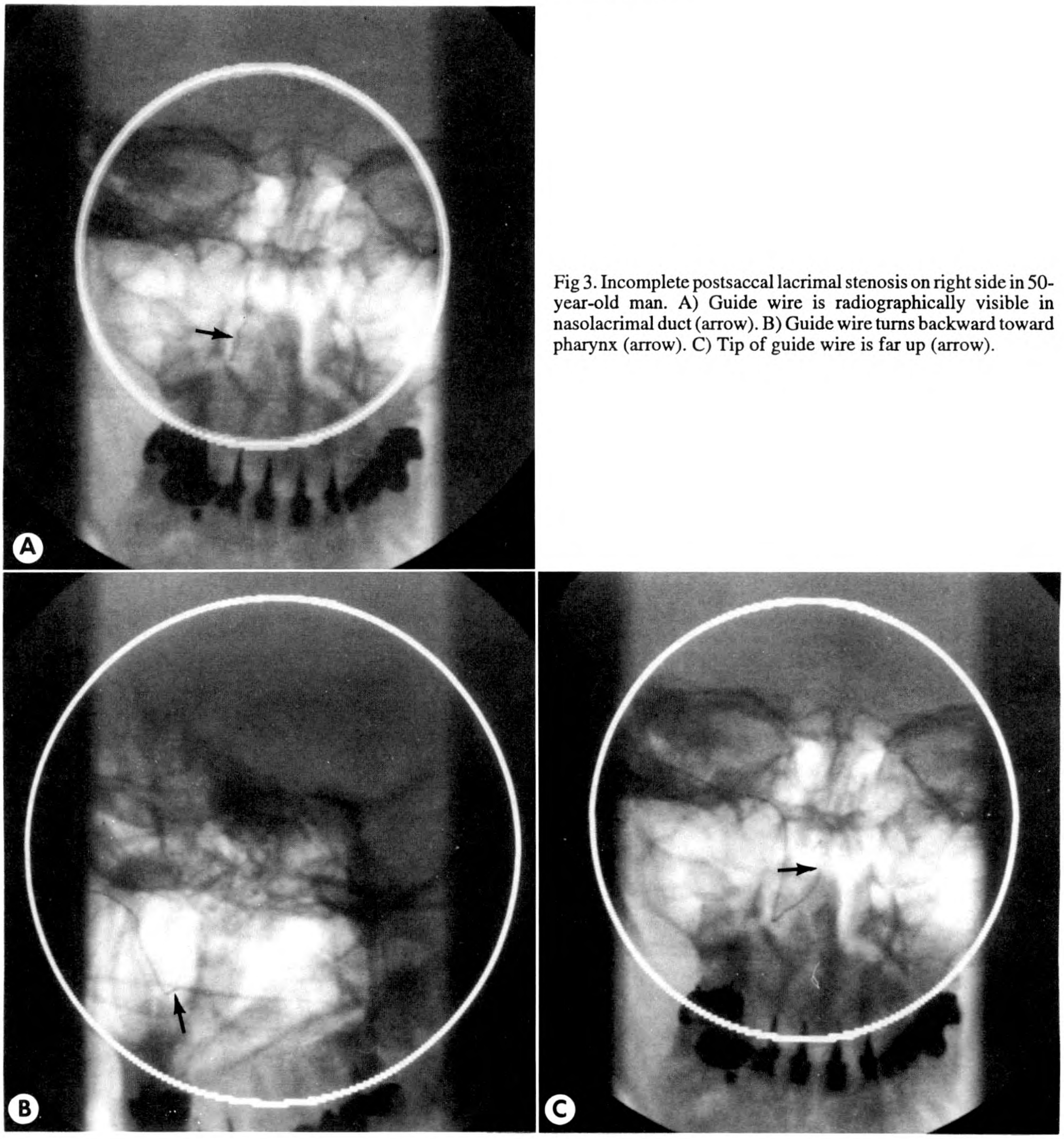

Fig 3. Incomplete postsaccal lacrimal stenosis on right side in 50-year-old man. A) Guide wire is radiographically visible in nasolacrimal duct (arrow). B) Guide wire turns backward toward pharynx (arrow). C) Tip of guide wire is far up (arrow).

follow-up time 22 months), all patients have been subjectively free of inflammation and complaint. At the last follow-up examination (February 1993) all eyes were dry, the silicone tubes had all been removed, the dye disappearance test was positive, and syringing of the lacrimal pathways was positive. In only 1 patient was a notable tendency to epiphora found in comparison to the healthy eye, and only as a result of exogenous irritation.

DISCUSSION

Ophthalmologists and rhinologists are often confronted with the symptom of epiphora. In some cases this poses a none too simple therapeutic problem. Especially in postsaccal stenosis of the nasolacrimal duct, the patient's sense of well-being is often greatly reduced, but more important, the risk of acute dacryocystitis is still prevalent. In the case of an infection of the upper respiratory tract, a total blockage of tear flow can result, caused by marked swelling of the nasal and lacrimal mucosa. In particular, those patients with stabilized incomplete stenosis of the nasolacrimal duct exhibit this predisposition. To date, for these patients the only two alternatives were a pro-

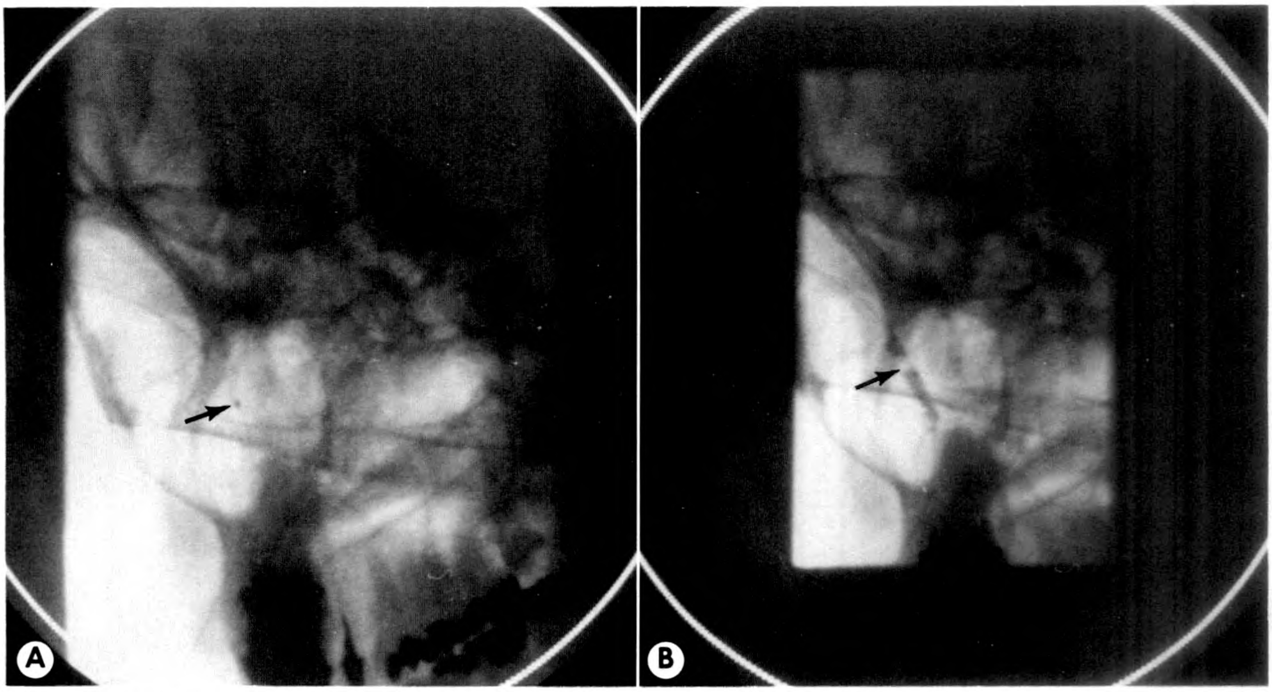

Fig 4. Same patient as in Fig 3. A) Exact placement of balloon catheter at site of stenosis by means of radiographic control (arrow). B) During dilation, stenosis is radiographically visible as substance defect of contrast medium–filled balloon (arrow).

phylactic local treatment with antiseptic and antibiotic eyedrops, or a surgical procedure such as external or endonasal dacryocystorhinostomy,[2-6] which had to be performed in the inflammation-free intervals.

With this new, noninvasive method of treatment, retrograde postsaccal balloon dilation, patients with incomplete stenosis of the nasolacrimal duct can undergo causal therapy. Dilation with a PTCA balloon catheter to open restenosis of postdacryocystorhinostomy anastomosis has been reported by Becker and Berry.[9] They have successfully used this technique in 5 children with congenital obstructions of the nasolacrimal duct, and also after unsuccessful probing or silicone intubation.[10] A group of radiologists[11] first used this method for postsaccal stenosis in adults. They performed only the balloon dilation, and did not stabilize the new opening with subsequent silicone intubation.[11] This seems to be of particular importance, as adhesions and scarring can occur postoperatively after irritation by instruments, damage of mucosa by dilation, and mechanical alteration of tissue.

PATIENT FOLLOW-UP TIME

Patient	*Sex*	*Operation Date*	*Follow-up Time (mo)*
1	F	Nov 1990	27
2	M	Nov 1990	27
3	F	Dec 1990	26
4	F	Jan 1991	25
5	M	May 1991	21
6	F	Aug 1992	6

When extensive mechanical opening of the postsaccal lacrimal passage is necessary, the guide wire cannot be introduced easily enough into the lacrimal sac and the nasolacrimal duct, so this method cannot be successful. It also cannot be used for true intrasaccal stenosis, as our 3 failed cases taught us.

Without long-term observation, permanent success cannot be proved. Within the follow-up period of 6 to 27 months, this new method of treatment seems to be able to achieve good results if the indication for retrograde balloon dilation is strictly limited to incomplete postsaccal stenosis, diagnosis of which requires digital dacryocystography.

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31ST WORKSHOP ON INNER EAR BIOLOGY

The 31st Workshop on Inner Ear Biology will be held in Montpellier, France, September 10-13, 1994. For further information, please contact Dr Guy Rebillard, INSERM U-254, Laboratoire de Neurobiologie de l'Audition, CHU Hopital St Charles, 34295 Montpellier cedex 05, France; telephone (33) 67 33 69 75; fax (33) 67 52 56 01.

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FIRST INTERNATIONAL SYMPOSIUM ON INNER EAR NEUROPHARMACOLOGY

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HISTOCYTOCHEMISTRY OF GLYCOCONJUGATES IN NASAL INVERTED PAPILLOMA

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In order to investigate the changes in cellular distribution of the glycocalyces in nasal inverted papilloma, formalin-fixed, paraffin-embedded biopsy specimens of inverted papilloma were analyzed by the avidin-biotin-peroxidase complex technique for the demonstration of peanut agglutinin (PNA) receptors, concanavalin A (*Canavalia ensiformis* agglutinin; ConA) receptors, carcinoembryonic antigen (CEA), and keratin, and compared with normal nasal mucosa, nasal polyps, and papillary adenocarcinoma. The inverted papillomas were positive for PNA and CEA, to the same degree as papillary adenocarcinoma. Their PNA binding was related to the degree of dysplasia. The ConA reaction was intermediate between that of normal mucosa and adenocarcinoma. The results suggest that the alteration of cellular glycoprotein structure in inverted papilloma is associated with its biologic characterization.

KEY WORDS — glycoconjugate, nasal inverted papilloma.

INTRODUCTION

Inverted papilloma is an unusual and special tumor of the head and neck, with disparate characteristics, ie, benign pathologic morphology and malignant clinical behavior. From the histologic picture, it is difficult to tell whether the tumor will become malignant.[1,2] Its cause is unknown.

The cellular membrane glycoproteins play an important role in cellular biologic specificity. In the process of cellular malignant transformation, a pathologic condition that alters the normal intercellular synthetic and catabolic processing may also result in the accumulation of specific glycoproteins and glycolipids, and change the glycoprotein structure in both cytoplasmic and membrane fractions.[3] Therefore, the use of lectins as histochemical probes may demonstrate the heterogeneity of the cellular surface glycocalyx in cancer cells, and herald invasive behavior in some tumors.[4]

In this study, peanut agglutinin (PNA), concanavalin A (*Canavalia ensiformis* agglutinin; ConA), carcinoembryonic antibody, and keratin antibody were used to show changes in glycoprotein structure on the cellular membrane of inverted papilloma.

MATERIALS AND METHODS

Twenty specimens of normal nasal mucosa (13 from men, 31 to 45 years of age, average 37.4 years; 7 from women, 29 to 43 years of age, average 34.6 years) were obtained from biopsy specimens that showed no pathologic abnormality. Surgically resected specimens from 40 cases of nasal polyps (28 from men, 24 to 67 years of age, average 41.7 years; 12 from women, 27 to 58 years of age, average 43.6 years), 40 cases of inverted papilloma (31 from men, 34 to 56 years of age, average 42.7 years; 9 from women, 37 to 62 years of age, average 47.1 years), and 30 cases of papillary adenocarcinoma (22 from men, 32 to 63 years of age, average 46.4 years; 8 from women, 41 to 53 years of age, average 45.3 years) were obtained. All tissues were fixed in formalin, embedded in paraffin, and cut into 50-µm-thick serial sections. One of them was stained with hematoxylin and eosin. The biotinylated lectins were purchased from Vector Laboratories Inc (USA); CEA from Dako Inc (USA); and keratin from Biosynthetic Laboratories, Beijing, China.

The entire procedure was carried out at room temperature. Sections were deparaffinized and rehydrated in the standard fashion and washed 3 times in phosphate-buffered saline (PBS; 0.1 mol/L phosphate 1.5 sodium chloride, pH 7.4). They were incubated in one of the lectins (10 µg/mL) for 60 minutes, rinsed in PBS as described above, incubated with 1:100 avidin-biotin-peroxidase complex reagent for 60 minutes, PBS-rinsed, counterstained with 1% methyl green in methanol, washed, dehydrated, cleared, and mounted. In a parallel experiment, lectin binding was inhibited by preincubation of each lectin with its appropriate binding sugar to confirm the specificity of lectin binding.

Sections reacted routinely with carcinoembryonic antibody and keratin antibody.

From the Departments of Otolaryngology (Huang, Jing, Li, Zhou) and Pathology (Ma), Changhai Hospital, Shanghai, People's Republic of China.

TABLE 1. RESULTS OF TEST FOR PEANUT AGGLUTININ RECEPTORS

Tissue	No. of Specimens	No. Positive
Normal nasal mucosa	20	0
Nasal polyp	40	0
Inverted papilloma	40	36
Papillary adenocarcinoma	30	30

RESULTS

All of the normal nasal mucosae and nasal polyps showed a complete lack of PNA (Table 1). Binding to PNA was seen in 36 cases (90%) of inverted papillomas and was related to their degree of dysplasia. Staining was stronger in the neoplastic cells with active dysplasia than in those with inactive dysplasia ($p < .01$; Table 2). The criteria for active dysplasia were as follows. Under observation with a light microscope, the cells of an inverted papilloma were within the scope of benign neoplasm, with cell and nuclear enlargement, greater pleomorphism, nuclear hyperchromatism, more mitotic activity, increase of the nuclear to cytoplasm ratio, cell disarrangement, and a lack of normal polarity. In papillary adenocarcinomas, 30 cases (100%) showed a strong PNA reaction. From normal nasal mucosa to papillary adenocarcinoma, ConA binding was progressively stronger ($p < .01$), and the staining granules increased from fine to coarse (Table 3). Binding to carcinoembryonic antigen (CEA) was negative in normal nasal mucosa and nasal polyps, but positive in inverted papillomas and papillary adenocarcinoma. Keratin binding was positive in all specimens (Table 4).

DISCUSSION

Inverted papilloma might be regarded as the most important and interesting of the tumors of the nasal region. The dominant histologic feature of the tumor is intensive proliferation of the tumor epithelium, with extensive invasion of the hyperplastic epithelium into the underlying stroma; it shows bone destruction by pressure atrophy without actual infiltration. Because of its high rates of recurrence (50% to 70%) and malignant change (3% to 24%), a pathologist can identify a given papilloma as benign or malignant, but cannot predict its clinical behavior. Some authors consider it a borderline or premalignant tumor.[5]

TABLE 2. REACTIVE INTENSITY OF PEANUT AGGLUTININ IN CELLS OF INVERTED PAPILLOMAS WITH DIFFERENT DEGREES OF DYSPLASIA

Degree of Dysplasia	No. of Specimens	Reaction			
		Strong	Moderate	Mild	None
Active	14	8	4	1	1
Inactive	26	1	5	13	7

TABLE 3. RESULTS OF TEST FOR CONCANAVALIN A RECEPTORS

Tissue	No. of Specimens	Reaction			
		Strong	Moderate	Mild	None
Normal nasal mucosa	20	0	3	9	8
Nasal polyp	40	2	8	14	16
Inverted papilloma	40	11	14	7	8
Papillary adenocarcinoma	30	12	9	7	2

The cellular membrane glycocalyx plays an important role in the neoplastic functions related to cellular antigenicity, contact regulation of cellular growth, invasiveness, evasion of immunologic detection, and ability to metastasize.[6] Biochemical, histochemical, and immunohistochemical studies have demonstrated differences between the glycoconjugates of normal mucosa and those of carcinoma.[7,8] The total sugar content in the membrane and cytoplasmic fractions is reduced in cancer tissues, with the variable activity of glycoprotein-glycosyltransferases, some showing no significant change and others being significantly reduced in cancerous tissues.[9] When some terminal sugar residues of a cellular membrane glycocalyx are deleted, the lectin receptors of subterminal sugar residues are increasingly unmasked. Thus, it would seem possible for lectins to identify the specific accumulated glycoproteins histochemically by demonstrating an intracellular accumulation of particular terminal sugar residues.

In this study, PNA reaction was negative in the cells of normal nasal mucosa and nasal polyps, but positive in the cells of inverted papillomas (90%) and nasal papillary adenocarcinomas (100%). In inverted papilloma, the PNA reaction was related to the degree of dysplasia, which progressively became stronger when dysplasia increased. Peanut agglutinin had a high specificity for the disaccharide B-D-Gal (1-3)-D-Gal NAc. The cellular glycoproteins were usually sialated — that is, they contained sialic acid as the terminal residue on the glycoprotein side chain. The PNA receptors found in inverted papillomas and papillary adenocarcinomas implied the presence of

TABLE 4. RESULTS OF TESTS FOR CARCINOEMBRYONIC ANTIGEN AND KERATIN

Tissue	No. of Specimens	No. Positive for Carcinoembryonic Antigen	No. Positive for Keratin
Normal nasal mucosa	8	0	8
Nasal polyp	8	0	8
Inverted papilloma	8	8	8
Papillary adenocarcinoma	8	8	8

exposed galactose at the end of the glycoprotein side chain, which would suggest some degree of desialation.[10] Similar differences between normal tissue and benign tumors or carcinomas have been described in the breast,[11] urinary bladder,[12] stomach,[13] parotid gland,[14] and other organs. Boland et al[15] considered that PNA-positive adenomata might have an increased risk of malignant transformation.

The ConA reactivity progressively intensified and the staining granules increasingly enlarged from normal nasal mucosa to papillary adenocarcinomas. Inverted papillomas showed a lectin-binding pattern that was intermediate between those of normal mucosa and carcinoma, which could be due to a loss of glycosyltransferases and a decrease in residual carbohydrate attached to the "stem" carbohydrate chains in the process.[16] Previous reports had demonstrated progressive loss of glycoconjugates during malignant transformation in other human organs, and changes in expression might be predictive of clinical tumor recurrence and progression.[17,18]

The structure of CEA and keratin was glycoprotein. The CEA was situated on the cellular surface. It was originally extracted from human fetal intestine and adult colon cancer tissue in 1965 by Gold and Freeman,[19,20] and is also present in tumors of other sites and in small amounts in nonmalignant tissues. Nevertheless, that 60% to 75% of all patients with colon cancer in one study had elevated circulating levels of the antigen and rising CEA levels suggests recurrence after resection for colon carcinoma.[21] In our study, the cells of normal nasal mucosa and nasal polyps were negative for CEA, but the cells of inverted papillomas and papillary adenocarcinomas were CEA-positive. All of them were positive for keratin. The results show that the cellular membrane glycoproteins of inverted papillomas changed as did those of papillary adenocarcinomas.

In conclusion, inverted papillomas showed the same changes in PNA receptors and CEA as did papillary adenocarcinoma, and a level of ConA binding intermediate between those of normal mucosa and carcinoma. This finding suggests that inverted papilloma might represent either a very early stage in the malignant process or reflect a change in cellular function that is an essential part of that process.

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LYMPHOCYTE MIGRATION TO THE MIDDLE EAR MUCOSA

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We investigated the migration of antigen-specific IgA–forming cells to the middle ear mucosa. Antigen-specific lymphocytes of IgA and IgG classes were induced in guinea pigs according to an immunization strategy previously described. From those animals, chromium 51–labeled lymphocytes of Peyer's patches and spleen were transferred to radiated chimera recipients. The radioactivity levels of the middle ears with antigenic and nonantigenic stimuli were significantly higher than those of the control ears ($p < .05$). Those levels of radioactivity were influenced neither by origins and subsets of transferred cells nor by antigenic stimuli to the mucosa ($p > .05$). Many labeled cells were observed in the middle ear effusion, while few were found in the inflamed mucosa. These findings suggest that in the early stage of inflammation, lymphocytes, including antigen-specific T and B cells, may be recruited from the blood circulation to the inflamed middle ear mucosa by nonspecific inflammatory processes that may mask antigen-specific factors in lymphocyte migration.

KEY WORDS — antigen-specific IgA–forming cells, chromium 51–labeled lymphocytes, lymphocyte migration, middle ear mucosa, radiated chimera.

INTRODUCTION

The middle ear mucosa, as well as the respiratory and gastrointestinal mucosae, has been demonstrated to possess immunologic features similar to those of peripheral mucosal sites in the common mucosal immune system.[1,2] After the enhancement of mucosal immunity, antigen-specific immunoglobulin A (IgA)–forming cells appear in the inflamed mucosa of the middle ear cavity and eustachian tube and play an important role in the local immune defense on the mucosa surface. These IgA plasma cells in the middle ear mucosa are thought to be derived from mucosa-associated lymphoid tissue (MALT) such as gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT).[3] These cells, originating from MALT, migrate via the draining lymphatics and thoracic lymphatic duct to the blood circulation and extravasate into the mucosal sites from the blood circulation. Recent investigations have demonstrated that various adhesion molecules are present on endothelial cells of high endothelial venules (HEVs), and lymphocytes possess surface receptors for these endothelial adhesion molecules.[4-8] These interactions between lymphocyte receptors and adhesion molecules are thought to regulate the lymphocyte extravasation. However, these interactions do not account for differences in retention and accumulation of lymphocytes in various tissues after extravasation. Recent studies suggest that receptor-ligand interaction between lymphocytes and glandular tissues, which is different from lymphocyte-HEV interaction, mediates the localization of IgA lymphocytes to the gland.[9,10] Our previous study[11] also suggests that the middle ear mucosa may possess organ-specific mucosal determinants with which B lymphocytes selectively bind.

Previous studies[12-14] suggest that the extravasation and selective migration of antigen-specific IgA lymphocytes into the mucosal sites are antigen-independent, while the antigen on the mucosal surface may produce only local persistence and retention of those cells. Furthermore, T lymphocytes have been demonstrated to be present between epithelial cells of the gut mucosa,[15-17] and it is suspected that they play a role in IgA plasma cell localization in the mucosa, possibly by producing a cytokine conductive to maturation and/or proliferation of extravasated IgA plasma cell precursors. However, the precise mechanism of selective migration and localization of antigen-specific IgA–forming cells in the middle ear mucosa is still unclear. In an effort to clarify the mechanism of migration of those lymphocytes to the middle ear mucosa, we investigated antigenic effects and influences of T cells from various lymphoid tissues on lymphocyte migration into the middle ear mucosa by using a reconstitution technique of lymphocytes in radiated chimera recipients of guinea pigs.

MATERIALS AND METHODS

Animals. Healthy male Hartley guinea pigs (4 to 6 weeks old; body weight 300 to 400 g) were used in this experiment.

Preparation of Antigen. Dinitrophenylated ovalbumin (DNP-OVA) was prepared by the method of Eisen et al[18] and conjugated with formalin-killed *Streptococcus mutans* strain 6715 (DNP-OVA–*S mutans*),

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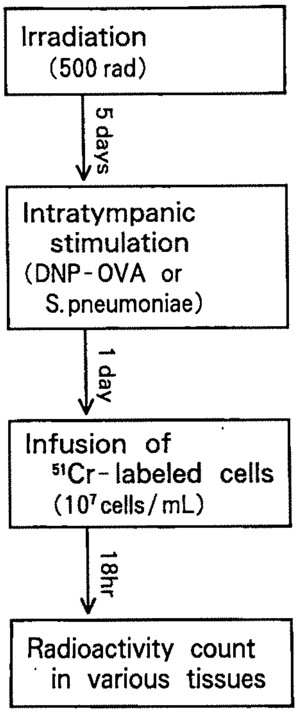
Irradiation
(500 rad)
5 days
Intratympanic stimulation
(DNP-OVA or S.pneumoniae)
1 day
Infusion of ^{51}Cr-labeled cells
(10^7 cells/mL)
18hr
Radioactivity count in various tissues

Fig 1. Experiment schedule.

as a particulate form of DNP-OVA antigen, according to the method described by Ternynck and Averameas.[19] The DNP-OVA antigen was determined to have 7 DNP groups per ovalbumin molecule by optical density analysis, and DNP-OVA–*S mutans* had 3 mg of DNP-OVA per 1 mg of bacterium.

Antisera. Antisera of rabbit against α- and γ-chains of guinea pig (ICN Immunobiologicals, Lisle, Ill) and goat anti rabbit immunoglobulin G (IgG) antisera conjugated with horseradish peroxidase and alkaline phosphatase (Cappel, Novern, Pa) were purchased from commercial sources.

Induction of Antigen-Specific IgA and IgG Antibody–Forming Cells. Guinea pigs were immunized with DNP-OVA for the induction of antigen-specific IgA and IgG antibody–forming cells according to an immunization strategy previously described.[3]

In order to elicit antigen-specific IgA–forming cells, animals were intraduodenally immunized with 200 μg of DNP-OVA coupled with *S mutans* strain 6715 in 200 μL of 0.01 mol/L phosphate-buffered saline 1 week after systemic priming with 200 μg of DNP-OVA mixed with Freund's complete adjuvant. In contrast, a systemic booster with 200 μg of DNP-OVA was carried out 1 week after priming to elicit antigen-specific IgG–forming cells. Two weeks after priming, saliva and serum samples were collected from these animals, and antigen-specific antibody titers of IgA and IgG in the samples were determined by an indirect enzyme-linked immunosorbent assay (ELISA) previously described.[3] In 14 animals with intraduodenal immunization, IgA antibody titers in saliva rose more than 2^4, while serum IgG antibody titers of 16 animals with systemic booster reached more than 2^6. From those animals with high antibody titers of IgA and IgG classes, Peyer's patches and spleens were collected as sources of antigen-specific IgA– and IgG–forming cells, respectively.

Preparation of Lymphocytes. Peyer's patches and spleens were collected from donor animals by laparotomy under anesthesia. Single cell suspension was prepared from these tissue samples by mincing and gently pressing between slide glasses. Following filtration through cotton wool, erythrocytes in the single cell suspension were lysed with 0.85% ammonium chloride, and the cells were washed 3 times in medium RPMI 1640 supplemented with 5% fetal calf serum. Finally, cells were resuspended at 1 × 10^8 cells per milliliter in the medium. T and B cells were isolated from the cell suspension by filtration through a nylon wool column according to the method of Julius et al.[20] One milliliter of 1 × 10^8 cells per milliliter suspension was applied to the column (Wako-ujunyakukougyo KK, Osaka, Japan) and incubated at 37°C for 45 minutes. The nonadherent cells were eluted dropwise at a rate of 3 mL/min to a total volume of 5 mL and collected as a T cell–enriched lymphocyte population. After 3-times washing of the column, the adherent cells were eluted by 10 mL of the medium pressed through the column and collected as a B cell–enriched lymphocyte population.

Detection of Antigen-Specific Antibody–Forming Cells of IgA and IgG Classes in Peyer's Patches and Spleen. Antibody-forming cells of IgA and IgG classes against DNP-OVA in Peyer's patches and spleen were detected by the solid-phase immunoenzymatic technique according to the method of Sedgwick and Holt.[21] Anti–guinea pig α- or γ-chain antiserum, as the first antibody, and alkaline phosphatase–conjugated anti-rabbit IgG antiserum of goat, as the second antibody, were used in this indirect ELISA plaque-forming assay, previously described.[3]

Labeling of Lymphocytes. Lymphocytes were labeled with chromium 51 (^{51}Cr) for an in vivo lymphocyte migration assay according to the method of Brunner et al,[22] and with tritium-labeled thymidine for autoradiography according to the method of Mitchell.[23] The 10^7 cells per milliliter lymphocyte suspension in RPMI 1640 was incubated with 100 μCi of ^{51}Cr or 10 μCi of tritium-labeled thymidine (Amersham Japan Ltd, Tokyo, Japan) at 37°C for 1 hour under 5% carbon dioxide. After 5-times washing, labeled cells were resuspended at 10^7 cells per milliliter in the medium.

Experiment Schedule. Figure 1 illustrates the ex-

TABLE 1. NUMBER OF ANTIGEN-SPECIFIC ANTIBODY–FORMING CELLS OF IGA AND IGG CLASSES IN PEYER'S PATCHES AND SPLEEN

		Antigen-Specific Antibody–Forming Cells in 10^5 Lymphocytes	
		Peyer's Patches	*Spleen*
Intraduodenal immunization (n = 14)	IgA	93 ± 15^{a}	13 ± 5^{e}
	IgG	20 ± 7^{b}	23 ± 8^{f}
Systemic immunization (n = 16)	IgA	8 ± 3^{c}	7 ± 4^{g}
	IgG	6 ± 3^{d}	225 ± 41^{h}

$a > e$, $a > c$, $a > g$: $p < .005$.
$h > d$, $h > f$, $h > b$: $p < .005$.

periment schedule of this study. Eighty-nine animals, as recipients, were exposed to 5 Gy of whole body γ-irradiation by using Gammacell 40 (cesium 137, AECL, Ontario, Canada) at the radioisotope center of Oita Medical University. Five days after irradiation, peripheral white cells of the animals declined to less than 1,000 cells per cubic millimeter.

Five days after irradiation, 69 recipient animals were inoculated into the right tympanic cavity with 500 µg of DNP-OVA in 100 µL of 0.8% hydroxypropyl cellulose solution. The left ear, as a control ear, received an injection of 100 µL of 0.8% hydroxypropyl cellulose solution. Twenty animals received an intratympanic inoculation with 10^8 live bacteria of *Streptococcus pneumoniae* biotype 3 in 100 µL of saline to induce nonantigenic inflammation of the mucosa. Six days after irradiation, the recipient animals were intravenously injected with 1×10^7 ^{51}Cr-labeled cells in 1 mL of the RPMI medium through a lower-extremity vein. Eighteen hours after injection, animals were anesthetized and bled by cardiac puncture. Both bullae and various lymphoid tissues, including Peyer's patches, mesenteric lymph nodes, cervical lymph nodes, and spleen, were collected. Bullae were cleaned of all soft tissues, and the middle ear cavity was left intact. The samples were weighed, and their ^{51}Cr levels were measured by a gamma counter. The radioactivity level of each sample was expressed as a proportion of total injected radioactivity (% CPM).

Five animals injected with lymphocytes labeled with ^{3}H-thymidine were used for autoradiographic analysis, and various tissue samples from them were prepared as previously described by Mitchell.[23]

Statistical Analysis. For statistical analyses, Student's *t* test was employed in this study.

RESULTS

Antigen-Specific Antibody–Forming Cells of IgA and IgG in Peyer's Patches and Spleen. Table 1 illustrates the mean number of antigen-specific antibody–forming cells of IgA and IgG classes in Peyer's patches and spleen from donor animals measured by an indirect solid-phase ELISA plaque-forming assay. Although the intraduodenal immunization did

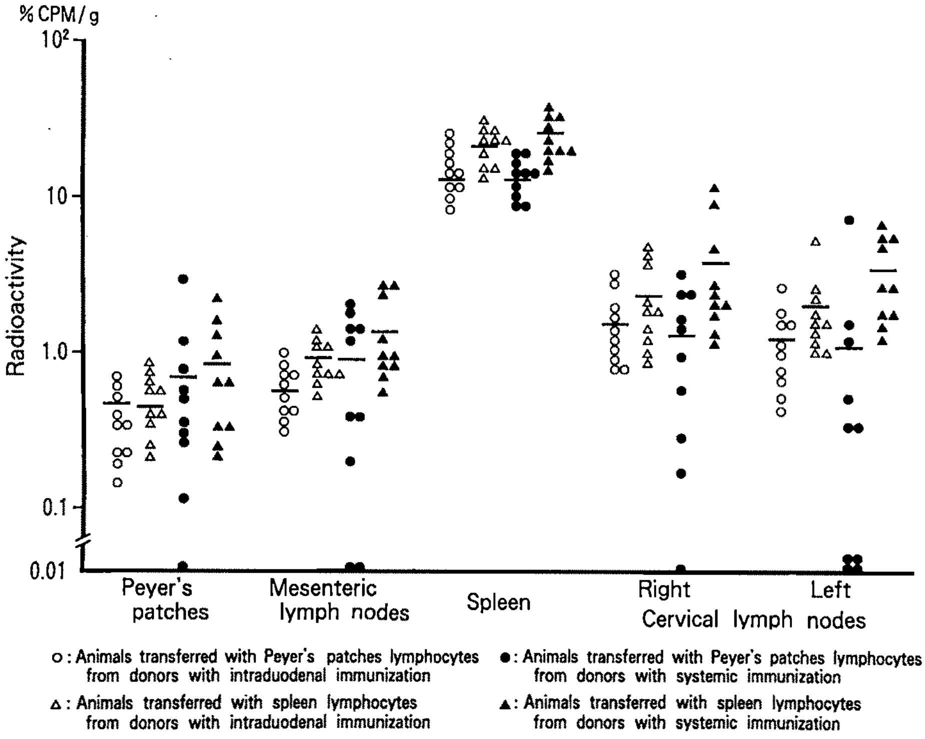

Fig 2. Distribution of chromium 51–labeled cells in various lymphoid tissues.

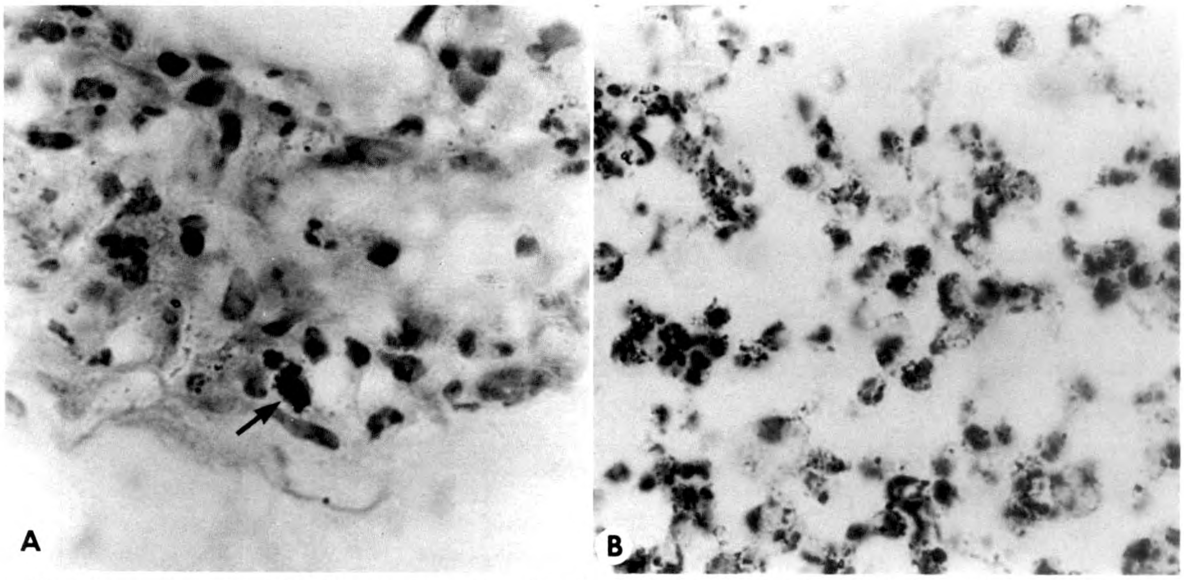

Fig 3. Autoradiographs. A) Middle ear mucosa. Some lymphocytes labeled with tritium-labeled thymidine (arrow) from Peyer's patches of animals with mucosal immunization are found scattered in inflamed mucosa. B) Middle ear effusion. Many labeled cells of Peyer's patches from animals with mucosal immunization are observed in effusion of recipients with nonantigenic stimulation on middle ear mucosa.

not elicit a significant increase in antigen-specific IgA–forming cells in the spleen ($p > .01$), it resulted in a remarkable increase of such cells in Peyer's patches from animals with mucosal immunization ($p < .005$). In contrast, the systemic immunization elicited a remarkable rise in antigen-specific IgG–forming cells in the spleen ($p < .005$), while those cells did not increase in Peyer's patches from animals without mucosal immunization ($p > .01$).

Distribution of ^{51}Cr-Labeled Lymphocytes in Various Lymphoid Tissues. The distribution of labeled lymphocytes in various lymphoid tissues, such as Peyer's patches, spleen, mesenteric lymph nodes, and cervical lymph nodes, is shown in Fig 2. Among the lymphoid tissues, the radioactivity levels in spleens of recipient animals are the highest, regardless of the origin of transferred lymphocytes and the immunologic condition of donor animals with or without mucosal immunization ($p < .01$). However, the distribution pattern of labeled lymphocytes in various lymphoid tissues of recipient animals was not influenced by the origin of transferred lymphocytes or the immunologic condition of donor animals with or without mucosal immunization ($p > .01$).

Lymphocyte Migration to Middle Ear Mucosa. As seen in Fig 3, labeled lymphocytes of Peyer's patches and spleen from animals with or without mucosal immunization were detected by autoradiography, and they were observed on the inflamed middle ear mucosa and in effusions from animals with antigenic and nonantigenic stimuli on mucosal surfaces of the middle ear. A few labeled cells were scattered in the lamina propria of the inflamed middle ear mucosa (Fig 3A), while many cells were observed in the middle ear effusions (Fig 3B).

Table 2 shows mean levels of radioactivity in the middle ear mucosae from donor animals with antigenic and nonantigenic stimulation to the middle ear. The mean levels of radioactivity in the middle ears with antigenic and nonantigenic stimulation were significantly higher than those of the control ears with neither antigenic nor nonantigenic stimulation ($p < .05$). The levels in the middle ears were not influenced by the origin of transferred lymphocytes, nor by the immunologic condition of donor animals with or without mucosal immunization ($p > .05$). The radioactivity levels in the middle ears of animals with antigenic stimulation were higher than those levels of the nonantigenic stimulation group, whereas these differences were not statistically significant among recipient animals with antigenic and nonantigenic stimulation to the middle ear ($p > .05$).

Table 3 shows mean levels of radioactivity in the middle ears stimulated with antigen after transfer of each fraction of labeled T and B cells. The mean levels of radioactivity in the middle ears were not significantly different among animals transferred with each fraction of T and B cells from Peyer's patches and spleen ($p > .05$). Those radioactivity levels of the middle ears were not influenced by the origin of transferred lymphocytes, nor by the immunologic condition of the donor animals with or without mucosal immunization ($p > .05$).

DISCUSSION

After the enhancement of mucosal immunity, an-

TABLE 2. RADIOACTIVITY OF MIDDLE EAR AFTER TRANSFER OF CHROMIUM 51–LABELED LYMPHOCYTES

			Radioactivity of Middle Ear (% CPM)	
		Sources of Lymphocytes	*Right*	*Left (Control)*
Intraduodenal immunization	Antigenic stimulation	Peyer's patches (n = 10)	0.198 ± 0.070*	0.106 ± 0.030
		Spleen (n = 10)	0.187 ± 0.076*	0.078 ± 0.030
	Nonantigenic stimulation	Peyer's patches (n = 5)	0.123 ± 0.030†	0.072 ± 0.012
		Spleen (n = 5)	0.103 ± 0.035†	0.038 ± 0.021
Systemic immunization	Antigenic stimulation	Peyer's patches (n = 10)	0.214 ± 0.159†	0.096 ± 0.061
		Spleen (n = 10)	0.213 ± 0.118†	0.093 ± 0.036
	Nonantigenic stimulation	Peyer's patches (n = 5)	0.121 ± 0.030†	0.101 ± 0.041
		Spleen (n = 5)	0.130 ± 0.072†	0.062 ± 0.010

CPM — count per minute. % CPM = (Radioactivity of each sample/Total injected radioactivity) ×100.

*$p < .01$.

†$p < .05$.

tigen-specific IgA–forming cells originating from MALT, such as GALT and BALT, are thought to migrate into the blood circulation via the draining lymphatics and thoracic lymphatic duct and extravasate into the inflamed mucosa of the middle ear cavity and eustachian tube from the bloodstream. Although the extravasation of lymphocytes may be regulated by the interaction between lymphocyte receptors and endothelial adhesion molecules of HEV, it is not clear that HEV and endothelial adhesion molecules are present in the middle ear mucosa. Recently, O'Sullivan and Montgomery[9] demonstrated the receptor-ligand interaction between lymphocytes and the lacrimal gland, which is different from the lymphocyte-HEV interaction studied by the method of in vitro adherence assay of lymphocytes. Thrane et al[10] found that clusters of IgA-producing cells were spatially related to human leukocyte antigen DR (HLA-DR)–positive ducts of the salivary gland and suggested that HLA-DR–expressing epithelium might contribute to local proliferation and terminal differentiation of IgA-producing plasma cells by glandular DR determinants via stimulation of helper T cells. These findings suggest that the interactions between lymphocytes and glandular tissues other than those between lymphocytes and HEV may regulate local retention and accumulation of IgA lymphocytes in the glands. Our previous study also showed that B lymphocytes from various lymphoid tissues, such as Peyer's patches and hilar lymph nodes, selectively bind with the inflamed mucosa of the middle ear as seen by in vitro adherence assay.[11] The inflamed middle ear mucosa may possess organ-specific mucosal determinants with which B lymphocytes selectively bind, and the lymphocyte binding with those mucosal determinants may regulate localization and local retention of IgA lymphocytes in the middle ear mucosa, as well as glandular tissues. As shown in this study, labeled lymphocytes were observed on the inflamed mucosa of the middle ear after transfer, while they are not detected on the middle ear mucosa without infection and inflammation. Therefore, those organ-specific determinants may be expressed on the middle ear mucosa by the inflammatory process.

Husband[12] demonstrated that the extravasation of antigen-specific IgA–containing cells into the gut lamina propria was antigen-independent, while these cells only persisted in the mucosal site if the antigen was administered on the mucosal surface. Ryan et al[13] suggested that lymphocyte migration into the middle ear mucosa from the blood circulation was independent of the origin of lymphocytes and antigenic stimulation on the mucosal surface of the middle ear. As in previous findings, the present study shows that lymphocyte migration into the middle ear mucosa was influenced by neither antigenic nor nonantigenic stimulation on the middle ear mucosa. Dunkley and Husband[14] also indicated that factors other than antigen are required for effective localization of IgA cells and suggested that T helper cells may play a role in IgA plasma cell localization in the

TABLE 3. RADIOACTIVITY OF MIDDLE EAR WITH ANTIGENIC STIMULATION AFTER TRANSFER OF CHROMIUM 51–LABELED T AND B LYMPHOCYTES

	Sources of Lymphocytes	*Radioactivity of Middle Ear (% CPM)*		
		T Cell Fraction	*B Cell Fraction*	*Whole Cell Fraction*
Intraduodenal immunization	Peyer's patches (n = 6)	0.138 ± 0.043	0.159 ± 0.048	0.152 ± 0.067
	Spleen (n = 6)	0.138 ± 0.046	0.132 ± 0.043	0.166 ± 0.046
Systemic immunization	Peyer's patches (n = 6)	0.175 ± 0.056	0.226 ± 0.058	0.278 ± 0.160
	Spleen (n = 6)	0.162 ± 0.048	0.239 ± 0.075	0.253 ± 0.143

CPM — count per minute. % CPM = (Radioactivity of each sample/Total injected radioactivity) ×100.

mucosa, possibly by producing a cytokine conductive to maturation and/or proliferation of extravasated IgA plasma cell precursors. Recently, intraepithelial lymphocytes have been demonstrated to be present in the epithelial layer of the gut mucosa, and their large population belongs to the T cells bearing T cell receptors.[15-17] The CD4+ and/or CD8+ T cell subsets of intraepithelial lymphocytes may affect full maturation and development of IgA plasma cell precursors originating in GALT by providing appropriate cytokines, including interleukin-5 and interleukin-6.[24] In contrast to the gastrointestinal mucosa, the normal middle ear mucosa with neither infection nor inflammation does not possess immune-competent cells that include intraepithelial lymphocytes, and it is protected against antigenic exposure by the eustachian tube.

Dunkley and Husband[25] also found that the migration of neither mature nor immature populations of T helper cells was significantly influenced by antigen after the transfer of antigen-specific T helper cells to syngeneic recipients. They suggest that antigen-specific T cells nonspecifically infiltrate the mucosal sites and may subsequently initiate rapid production of attractants for nonspecific cells, masking the effect of antigen-specific factors. As shown in this study, after the transfer of labeled lymphocytes, the radioactivity of the inflamed middle ear mucosa attributed to antigenic and nonantigenic stimuli was significantly higher than that of the normal mucosa without infection and inflammation. In contrast, the antigenic stimulation on the mucosal surface of the middle ear did not affect lymphocyte migration into the middle ear mucosa, even though antigen-specific IgA– and IgG–forming lymphocytes from Peyer's patches and spleen were transferred. Furthermore, as demonstrated by autoradiography, many labeled lymphocytes were found in the middle ear effusion from animals with middle ear inflammation due to antigenic and nonantigenic stimuli, while few labeled cells were observed scattered in the inflamed mucosa. These findings are consistent with previous results and suggest that in the early stage of the inflammation of the middle ear mucosa, circulating lymphocytes, including antigen-specific T and B cells, may be attracted and recruited from the blood circulation to the inflamed mucosa by nonspecific inflammatory processes that may mask antigen-specific immune processes in lymphocyte migration. In the early stage of the inflammation, various adhesion molecules may be expressed on the endothelial surface of the vessels in the inflamed local sites by lipopolysaccharide, tumor necrosis factor, and interleukins derived from bacteria and macrophages. Circulating lymphocytes bearing surface receptors may adhere to the endothelial surface by interactions between adhesion molecules and lymphocyte receptors. After trapping, lymphocytes may migrate to the junctions between endothelial cells and transendothelially enter the local site.

In order to clarify selective migration and retention of antigen-specific IgA–forming cells into the middle ear mucosa, further studies are needed. It is necessary to define the precise immunologic and immunochemical characteristics of organ-specific determinants and ligands on the middle ear mucosa that IgA B lymphocytes bind by receptors on their surface. The expression of these mucosal determinants and ligands on the middle ear mucosa should also be clarified.

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INFLUENCE ON RABBIT SUBMANDIBULAR GLAND INJURY BY STIMULATION OR INHIBITION OF GLAND FUNCTION DURING IRRADIATION

HISTOLOGY AND MORPHOMETRY AFTER 15 GRAY

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The loss of salivary gland function after a single dose of 15 Gy was correlated to loss of gland weight and degeneration of seromucous acini and serous tubules at 10 months postirradiation. The serous tubules were degranulated rather early, but regenerated after some months in an abnormal way as adenomatous structures. Striated ducts were mainly unaffected by irradiation. Arterioles showed slight to moderate narrowing of the lumina. There was an increased amount of plasma cells in the gland lobules at 10 months postirradiation. Histologic and morphometric criteria and changes in gland weight showed less pronounced radiation injury in glands irradiated during inhibition of the gland function by biperiden (Akineton) compared to glands irradiated during stimulation of the gland function by pilocarpine. This finding may offer a clinically important means to reduce salivary gland dysfunction after radiation treatment of tumors in the head and neck regions.

KEY WORDS — biperiden, pilocarpine, radiation, radioprotection, salivary gland histopathology.

INTRODUCTION

Radiotherapy for cancer in the oral cavity often includes the salivary glands and results in complications, including dryness of the mouth, loss of taste acuity, dental caries, and ulceration of the oral mucosa. Apart from pain and difficulty in eating, these effects may seriously compromise nutrition and oral health. To a great extent they stem from reduction of salivation due to radiation damage to the salivary glands.[1-6]

Although such effects of irradiation were observed early in the history of radiotherapy (1911) by Bergonié and Speder,[7] the relationships among time, dose, and pathologic changes in the usual clinical circumstances are not yet clearly understood. The response to radiation injury is a function of the radiosensitivity of various tissue components.[1,5,6] Although the salivary glands might be assumed to be relatively radioresistant, because of the normally low mitotic rate of their cells, the prompt effect of radiation on their function indicates these glands are highly radiosensitive.[1]

It is of clinical importance to find methods to reduce these disadvantageous side effects of radiation treatment. Our hypothesis is that the functional state of the glands during radiation has an effect on the degree of damage. The purpose of this paper is to report the impact on the radiation effects on salivary gland function produced by parasympathomimetic stimulation with pilocarpine or parasympatholytic inhibition with biperiden during radiation.

The rabbit submandibular gland is of the heterocrine type in which the secretory end piece is mainly built up of two morphologically and histochemically different structures: proximally, the serous tubules, and distally, the seromucous acini (Fig 1[8]). As presented in a previous paper, the trichrome staining of plastic sections was found to be superior in respect to resolution and clarity of morphological details and was well able to discriminate between the two different structures and granules building up the secretory end pieces.[8] We have described histologic and quantitative changes of the different tissue components of irradiated, untreated rabbit submandibular glands over time from the acute to the chronic phase.[9] The gland weight was reduced, as well as the gland lobules, seromucous acini, and serous tubules. The striated ducts showed no relevant signs of radiation damage. The number of plasma cells was increased, but capillaries and arterioles showed only slight to moderate fibrosis. The serous tubules regenerated, often as abnormal adenomatous structures.[9]

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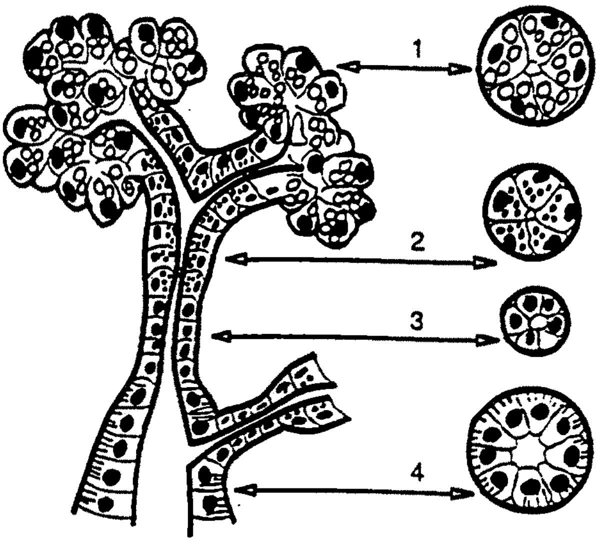

Fig 1. Schematic representation of rabbit submandibular gland secretory end piece. 1 — seromucous acinus, 2 — serous tubule, 3 — intercalated duct, 4 — striated duct. (Modified from Hagelqvist et al[8]).

MATERIALS AND METHODS

ANIMALS

Sixty healthy rabbits of both sexes of the New Zealand White strain were kept in separate hutches and had unrestricted access to food (rabbit pellets) and water.

Thirty rabbits constituted a control group with no exposure to radiation. Ten animals were sacrificed painlessly and examined at 3, 7, and 13 months of age, respectively.

A second group included 15 rabbits irradiated at 3 months of age. The rabbits received a parasympathomimetic agent, pilocarpine chloride, 0.1 mg/kg body weight, given intravenously 3 minutes before irradiation, in order to stimulate the salivary flow and gland function during irradiation. Eight rabbits were examined at 7 months of age, and 6 at 13 months of age. One rabbit died before the final examination.

A third group included 15 rabbits irradiated at 3 months of age. The rabbits received a parasympatholytic agent, biperiden lactate (Akineton, Knoll AG), 3 mg/kg body weight, given intravenously 2 minutes before irradiation, in order to inhibit the salivary flow and gland function during irradiation. Seven rabbits were examined at 7 months of age, and 6 at 13 months of age. Two rabbits died before the final examination.

IRRADIATION

Irradiation, at age 3 months, was performed with 4-megavolt x-rays (Philips SL 75/5). An axial beam was directed from above toward the rabbit's head, including the skull base and the tip of the nose. The focus skin distance was 100 cm, and the field size was 14 x 20 cm, with half of the field shielded by a 7-cm-thick lead block. During irradiation the rabbit was in a box with its head fixed. Either the left or the right half of the head was unshielded during irradiation, and the shielded side served as a reference. As a single field was used, the dose had to be specified at the depth of the organ. The position of the submandibular glands was calculated from radiographic and nuclear magnetic resonance imaging of 3 rabbits in the study. The mean depth from the top of the head to the glands was assessed as 5 cm. A mean absorbed dose of 15 Gy was given to the glands, which represents an absorbed dose of 18 Gy at depth dose maximum. A midline laser light beam was used to align the rabbit in the longitudinal axis, and opposing lateral lasers were used for horizontal alignment and focus skin distance.[9]

In order to verify the dose distribution and reproducibility of the irradiation, thermoluminescence dosimeters — lithium fluoride rods 1 mm in diameter and 6 mm long — were placed under the rabbit's mandible. The dosimeters were placed at 5-mm intervals up to 20 mm distance from the border line between the unshielded and the shielded fields. On the shielded side the dosimeters determined the dose gradient and the absorbed dose to the gland that served as a reference, while those on the unshielded side verified that the intended absorbed dose was given. Five separate measurements were made with the dosimeters. To illustrate the reproducibility, the measurements were made on 2 different rabbits on different occasions. The distribution of the absorbed dose was also determined by using a film placed in a polystyrene phantom at a depth corresponding to that of the submandibular glands.

The absorbed dose in the boundary plane between the left and right sides was 50% of the given dose. At 10 mm from the boundary line, the unshielded side received 97%, and the shielded side 12% of the delivered dose. Because of small positioning errors, the border line between the shielded and unshielded sides of the rabbits' heads did not always correspond to the anatomic median plane. This variation was estimated, and the mean doses to the unshielded and the shielded glands in a group of rabbits were calculated to be 87% and 18%, respectively, of the delivered dose. Consequently, a delivered dose of 15 Gy gave on average ≥13.1 Gy and ≤2.7 Gy, respectively, to the unshielded and shielded glands. A detailed description of the radiation performance and the dose geometry is separately reported.[9]

TISSUE PREPARATION

The rabbits' body weight was registered at 3, 7, and

TABLE 1. BODY WEIGHT AND TOTAL WEIGHT OF SUBMANDIBULAR GLANDS IN NONIRRADIATED CONTROL RABBITS AND OF SHIELDED AND UNSHIELDED GLANDS IN IRRADIATED RABBITS (PILOCARPINE OR BIPERIDEN PRETREATMENT) AT AGE 7 AND 13 MONTHS (4 AND 10 MONTHS POSTIRRADIATION)

		Nonirradiated			*Irradiated 15 Gy; Pilocarpine*				*Irradiated 15 Gy; Biperiden*		
						Gland Weight				*Gland Weight*	
Age (mo)	*N**	*Body Weight*	*Gland Weight*	*N**	*Body Weight*	*Shielded*	*Unshielded*	*N**	*Body Weight*	*Shielded*	*Unshielded*
3	10	2.4 ± 0.4	0.61 ± 0.17								
7	10	4.0 ± 0.5	0.78 ± 0.12	8	3.8 ± 0.2	0.65 ± 0.06	0.50 ± 0.07	7	3.9 ± 0.4	0.84 ± 0.16	0.66 ± 0.10
13	10	4.2 ± 0.9	0.85 ± 0.10	6	4.1 ± 0.8	0.63 ± 0.08	0.44 ± 0.19	6	4.8 ± 0.7	0.94 ± 0.14	0.63 ± 0.13

Data are mean ± SD in grams.

*Number at final examination.

13 months of age. The rabbits were sacrificed by intravenous injection of saturated potassium chloride. The submandibular glands were removed, weighed, and divided longitudinally into two equal parts. Each part was further divided into at least four equal pieces in order to get good fixation of the tissues. One half from each gland was fixed in Karnovsky's fixative[10] and embedded in glycol methacrylate (Historesin Embedding System, Leica Instruments GmBh, Germany) and cut with a glass knife into 2-μm sections.[8] The different sections were stained by the modified trichrome method of Crowder.[11]

MORPHOMETRY

For the morphometric analysis, a Leitz Ortholux microscope was used with a 40× lens and a 10× ocular with a grid divided into 100 squares (Leitz 1.0). In each examined section 20 close viewing fields (2,000 squares) were estimated.[9]

At least four different sections from each gland were examined at light microscopy and used for histochemical, morphological, and morphometric investigations. A detailed description of the histochemical and morphometric procedures is separately reported.[8,9]

The proportions of intralobular tissue were estimated. The relative surface of the seromucous acini, serous tubules, and striated ducts within the lobule was calculated as the percentage of the total intralobular tissue surface. These three major compartments were together computed as 100%, thus excluding fibrous tissue, fat, blood vessels, and excretory ducts.[9]

Weight by Percent. Statistical analysis of variance was applied to gland weight. The weights of the three major intralobular compartments could be assessed from calculations based on the figures for total gland weight, intralobular tissue as the percentage of total gland weight, and tissue components as the percentage of intralobular tissue weight (Tables 1 and 2).[9] The weight changes between 3 and 7 or 13 months of age were then presented as the percentage of the weight values at 3 months. The results illustrate the impact of irradiation on the normal growth of the different compartments.

RESULTS

GLAND WEIGHT

In the control group of nonirradiated rabbits the mean submandibular gland weight was 0.85 g at age 13 months. The unshielded glands with pilocarpine stimulation during irradiation weighed on average 0.44 g at age 13 months (10 months postirradiation), which was significantly less ($p < .05$) than the 0.63 g in the rabbits treated with biperiden (Table 1).

HISTOPATHOLOGY

The normal histologic appearance and the radiation injuries in glands with no pharmacologic treatment during irradiation are described in detail else-

TABLE 2. DISTRIBUTION OF MAJOR INTRALOBULAR COMPARTMENTS OF SUBMANDIBULAR GLANDS IN NONIRRADIATED AND IRRADIATED RABBITS WITH PILOCARPINE OR BIPERIDEN TREATMENT DURING IRRADIATION

		Pilocarpine				*Biperiden*			
		4 mo After 15 Gy		*10 mo After 15 Gy*		*4 mo After 15 Gy*		*10 mo After 15 Gy*	
Compartments	*Age 3 mo, Nonirradiated*	*Shielded*	*Unshielded*	*Shielded*	*Unshielded*	*Shielded*	*Unshielded*	*Shielded*	*Unshielded*
Seromucous acini	80.9 ± 1.3	79.7 ± 3.2	78.3 ± 2.9	83.3 ± 1.0	46.7 ± 36.9	81.6 ± 2.6	79.9 ± 5.5	83.5 ± 1.2	73.2 ± 5.6
Serous tubules	10.5 ± 1.0	11.1 ± 2.6	2.9 ± 2.0	8.1 ± 1.4	6.7 ± 9.0	10.4 ± 2.2	5.9 ± 3.0	8.9 ± 1.8	9.5 ± 6.9
Striated ducts	8.6 ± 0.4	9.2 ± 1.4	18.8 ± 3.5	8.6 ± 0.6	46.6 ± 41.3	8.0 ± 1.0	14.2 ± 3.1	7.6 ± 0.9	17.3 ± 8.4

Data are mean ± SD in percent.

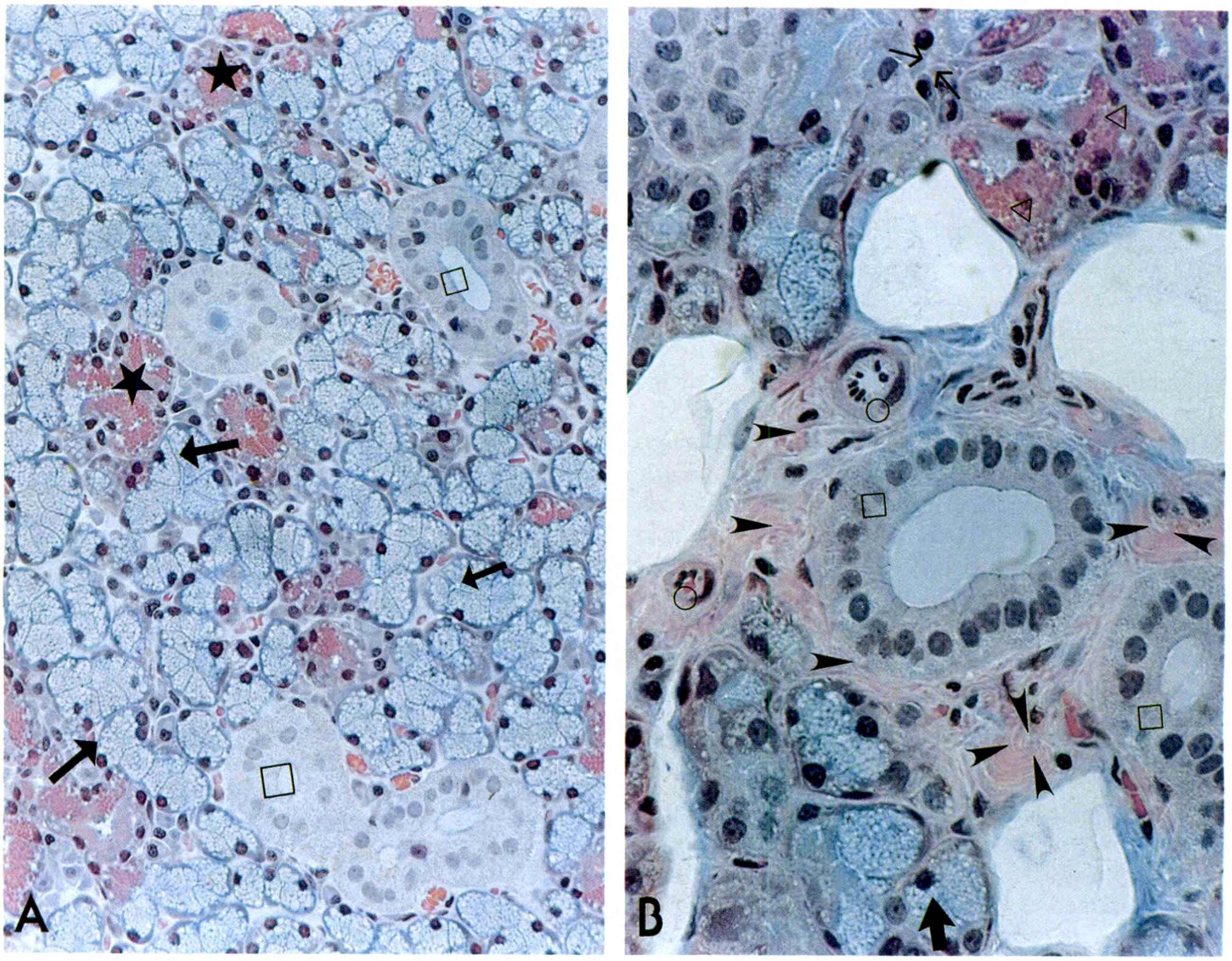

Fig 2. Rabbit submandibular glands. A) Normal histologic appearance (original ×280). Seromucous acini (arrows), serous tubules (stars), and striated ducts (squares). B) Ten months after 13.1 Gy of irradiation with no pharmacologic treatment (original ×375). Seromucous acini are partly atrophic, with very small granules (thick arrow). Serous tubules are regenerated and arranged in adenomalike pattern (open triangles). Around rather normal striated ducts (square) there is moderate fibrosis (closed triangles), where small arterioles (circles) with narrow lumina are seen. Small aggregations of plasma cells are seen between acini (thin arrows).

where[8,9] (Fig 2). The observed postirradiation changes in glands treated pharmacologically during irradiation were evaluated with reference to these earlier-reported appearances.

Pilocarpine-Treated, Unshielded Glands of Irradiated Rabbits. In the seromucous acini at 4 months postirradiation (7 months of age), a slight edema with dilated interacinic spaces and, in spots, considerable numbers of plasma cells were present. The acini differed in size and the granules were often small (Fig 3A). These signs were more pronounced at 10 months postirradiation, and the acini also showed a changed architecture with very small granules. In 1 animal there was even a total atrophy, and in another a very pronounced atrophy of the seromucous acini (Fig 3B).

At 4 months after irradiation an extensive degranulation and signs of pathologic regeneration with adenomatous structures were shown in the serous tubules in several animals (Fig 3A). At 10 months after irradiation, the degranulation and regeneration were even more pronounced. In 2 animals a complete degranulation and almost complete atrophy of the serous tubules were noticed. Only fibrous tissue and ducts were left, and signs of regeneration were not observed in these 2 cases. In the other 4 cases there was a very small amount of serous granules, which almost solely formed adenomatous structures. No serous tubules with a normal appearance were observed (Fig 3B).

At 4 months after irradiation, slightly swollen epithelial cells were the only signs of radiation effect in the striated ducts, but in a few animals there were dilated ducts in some regions. At 10 months postirradiation, 2 cases showed atrophic glands that except for fibrotic tissue were composed almost totally of striated ducts of different calibers. In the cytoplasm

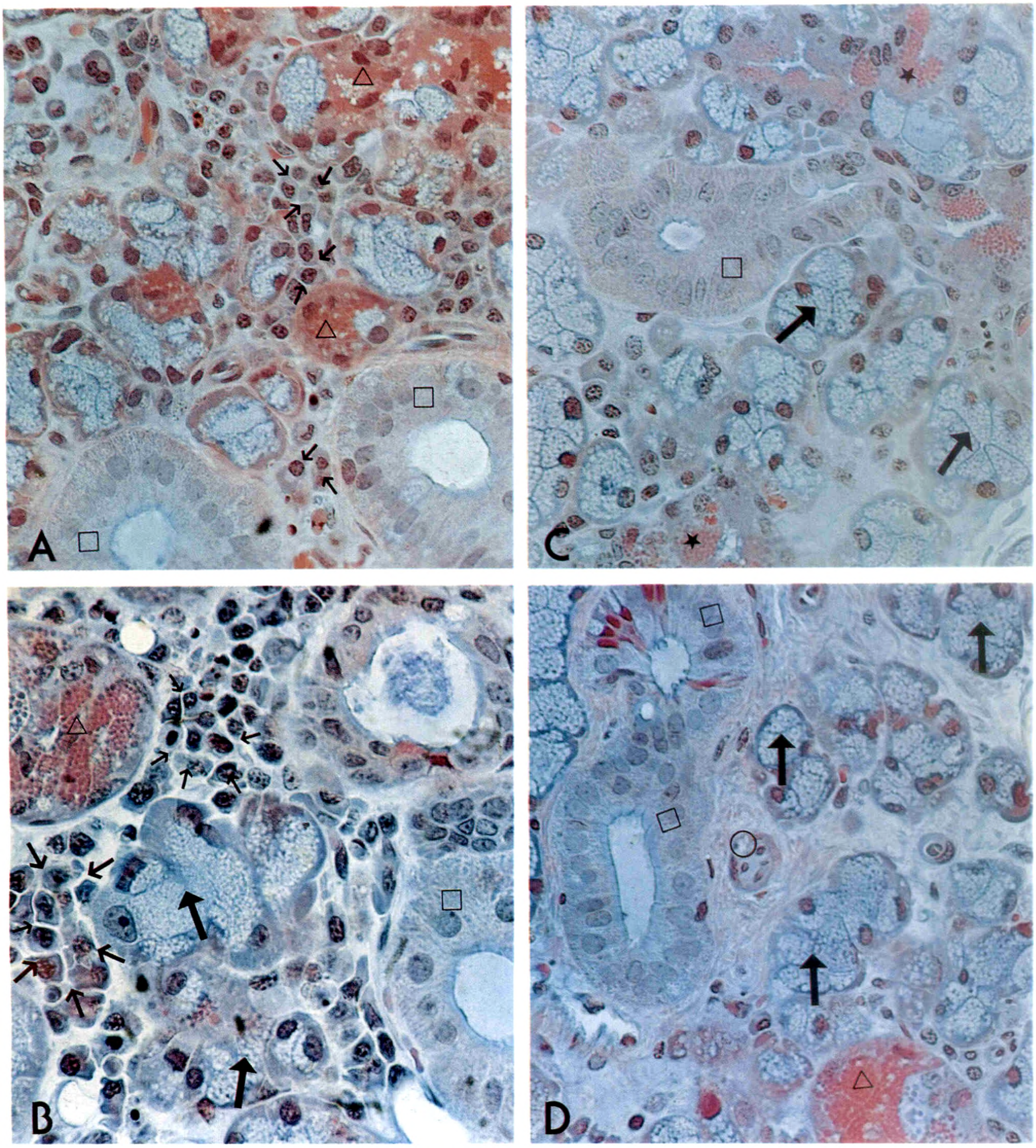

Fig 3. Pretreated submandibular glands (original ×500). A,B) Pilocarpine-pretreated. A) At 4 months postirradiation. Adenomalike regeneration of serous tubules is seen (triangles). Large numbers of plasma cells (arrows) are seen. Striation of striated ducts (square) is well preserved. B) At 10 months postirradiation. Large numbers of plasma cells (thin arrows) almost dominate viewing field. Seromucous acini (thick arrows) have changed architecture. Only few serous tubules, all with adenomalike appearance (triangle), are seen. Striation of striated ducts (square) is well preserved. C,D) Biperiden-pretreated. C) At 4 months postirradiation. Seromucous acini (arrows) are sometimes small, but almost unchanged. Some few serous tubules (star) with almost normal appearance are seen. Striation of striated ducts (square) is well preserved. D) At 10 months postirradiation. Seromucous acini (arrows) appear rather normal, but partly atrophied. Serous tubules with adenomalike structures (triangle) are seen. Around striated ducts (squares), moderate fibrosis encircles small arteriole (circle) with narrow lumen.

of the duct cells the striation was mostly well preserved. Some intercalated ducts were also seen (Fig 3A,B).

The arterioles, and in some cases also the capillaries, were somewhat thicker both 4 and 10 months after irradiation. At 10 months postirradiation, a moderate stasis was observed in the capillaries.

The amount of fibrous tissue was, at 4 months after irradiation, slightly increased around the wider striated ducts and the arterioles, and around some acini and in the hilus region. At 10 months after irradiation, the fibrosis was pronounced, especially in the 2 cases with severe atrophy.

The plasma cells were found in increased amounts in the edematous fluid and around the striated ducts, at both 4 and 10 months after irradiation (Fig 3A,B).

Where ganglion cells could be seen, they showed degenerative injuries with partly cytolyzed nuclei.

Biperiden-Treated, Unshielded Glands of Irradiated Rabbits. In the seromucous acini, at both 4 and 10 months postirradiation (7 and 13 months of age, respectively), slightly dilated interacinar spaces were observed. The mucous granules showed no signs of injury, but differed in size at both 4 and 10 months postirradiation. The acinar architecture was slightly changed in single acini at 10 months postirradiation, but most acini looked normal (Fig 3C,D).

At 4 months after irradiation, a reduced number of granules was noticed in the serous tubules. In some tubules the granules had disappeared, and in others they were small. No adenomatous regeneration was observed (Fig 3C). At 10 months postirradiation there were signs of atypical regeneration with adenomalike structures and intercellular vacuolization. However, quite normal tubules without any signs of radiation injury were found in spots (Fig 3D).

At both 4 and 10 months postirradiation no signs of injury, except slightly swollen cell nuclei, were seen in the striated ducts. However, in 1 case in which the gland was very atrophic, the cell nuclei of the duct epithelium were partly cytolyzed, and the nuclei varied in size. In this atrophic gland there were also many small ducts with the appearance of intercalated ducts.

The arterioles and also the capillaries had a normal appearance at both 4 and 10 months postirradiation. At 10 months there was a slight narrowing of the lumina of the arterioles. The capillaries showed generally a slight stasis at both 4 and 10 months postirradiation (Fig 3D).

The amount of fibrous tissue was unchanged at 4 months postirradiation, but was slightly increased around the acini and around single, wider striated ducts at 10 months postirradiation (Fig 3D).

Plasma cells were found in increased numbers in the extracellular fluid between the acini and around the striated ducts, especially at 10 months postirradiation.

Shielded Glands of Irradiated Rabbits. No significant difference in any histologic parameter was found between the shielded glands of irradiated rabbits and the control glands of the nonirradiated rabbits (Fig 2).

MORPHOMETRY

The gland weight was reduced by irradiation, and the proportions between different intralobular components were changed (Table 2). There were no relevant signs of radiation damage of the striated ducts in any group of submandibular glands. Therefore, it could be assumed that there was no difference in the number of striated ducts in any of the glands (ie, irradiated shielded and unshielded, and nonirradiated).

Weight by Percent of Pilocarpine-Treated, Unshielded Glands of Irradiated Rabbits. The rabbit body weight increased from 100% at 3 months of age to 158% at 7 months and to 170% at 13 months of age (Fig 4 and Table 1).

The shielded glands increased in weight from 100% at 3 months of age to 106% at 7 months and to 103% at 13 months of age, but the unshielded gland weight decreased from 100% at 3 months of age to 81% at 7 months and to 72% at 13 months of age (Fig 4).

The intralobular tissue of shielded glands showed almost no change from 100% at 3 months of age to 97% at 7 months and to 103% at 13 months of age, but that of the unshielded glands decreased to 48% at 7 months and to 19% at 13 months of age (Fig 4).

The seromucous acini of the shielded glands were almost unchanged from 100% at 3 months of age to 95% at 7 months and to 106% at 13 months of age. In contrast, the seromucous acini of the unshielded glands were reduced to 47% at 7 months and to 11% at 13 months of age (Fig 4).

The serous tubules of the shielded glands showed no growth (103%) at 7 months of age and a decrease in weight at 13 months to 79% (Fig 4). In the corresponding unshielded glands the serous tubules showed considerable reduction in weight to 14% at 7 months of age and to 12% at 13 months of age. There were no signs of regeneration.

The striated ducts increased in weight to 106% at 7 months of age and to 102% at 13 months of age, and in accordance with the presumption for this calculation, there was no difference between shielded and unshielded glands (Fig 4).

Weight by Percent of Biperiden-Treated, Unshielded Glands of Irradiated Rabbits. The rabbit

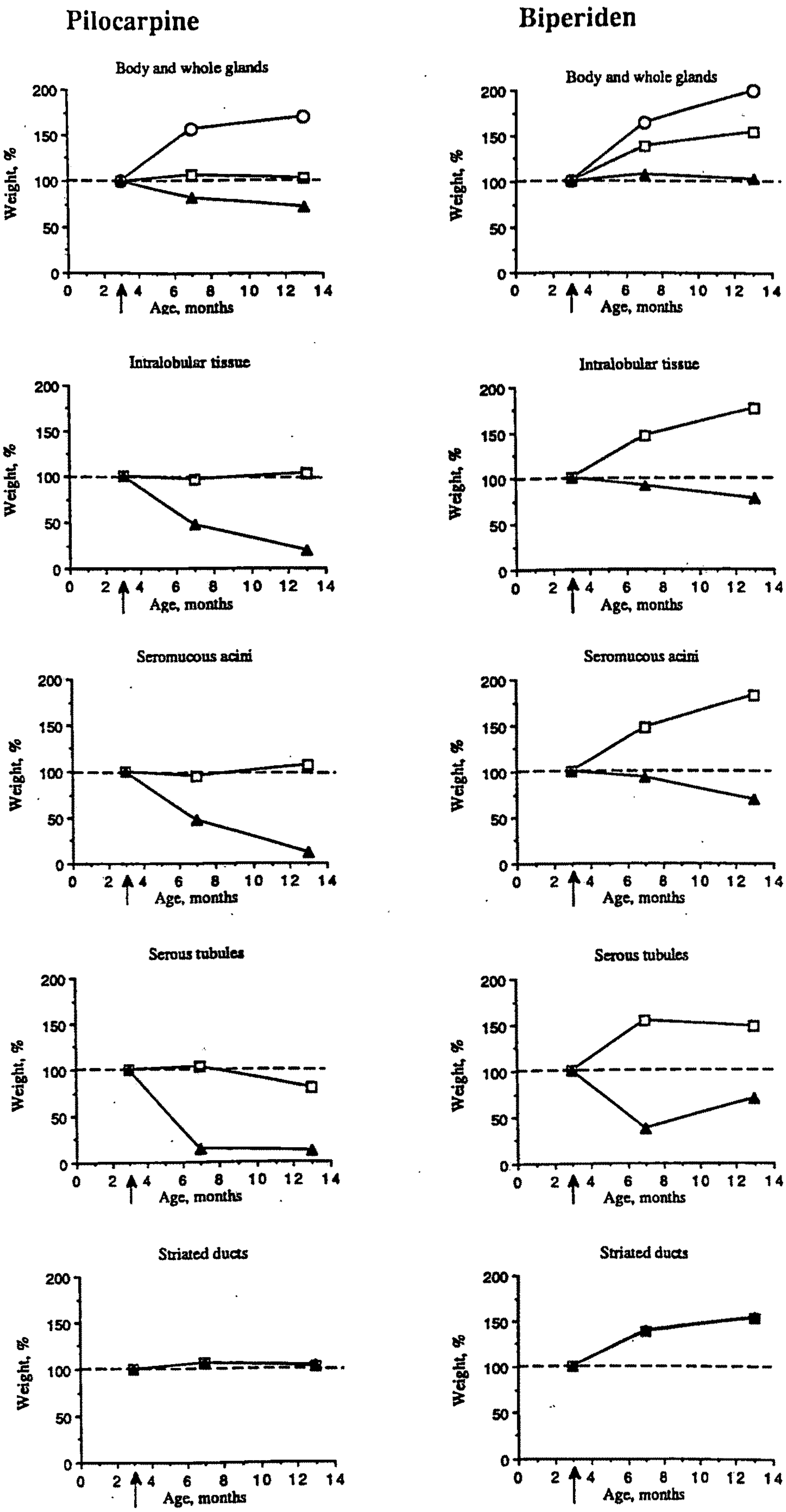

Fig 4. Weight growth following 15 Gy of irradiation. Relative weights are calculated as percentage of those in nonirradiated control group at age 3 months. (Arrows indicate time of irradiation.) Changes in rabbit body weight and whole submandibular gland, intralobular tissue, seromucous acini, serous tubules, and striated ducts after pilocarpine or biperiden treatment, respectively. Circle—whole body, square — shielded glands, triangle — unshielded glands.

body weight increased from 100% at 3 months of age to 164% at 7 months and to 199% at 13 months of age (Fig 4).

The shielded glands increased in weight to 138% at 7 months and to 154% at 13 months of age, but the unshielded gland weight increased from 100% at 3 months of age to 108% at 7 months and to 103% at 13 months of age (Fig 4).

The intralobular tissue of shielded glands showed increased weight from 100% at 3 months of age to 146% at 7 months and to 176% at 13 months of age, but that of the unshielded glands decreased to 91% at 7 months and to 77% at 13 months of age (Fig 4).

The seromucous acini of the shielded glands increased from 100% at 3 months of age to 147% at 7 months and to 182% at 13 months of age. In contrast, the unshielded glands were reduced to 93% at 7 months and to 69% at 13 months of age (Fig 4).

The serous tubules of the shielded glands increased to 154% at 7 months of age and to 148% at 13 months (Fig 4). In the corresponding unshielded glands, the serous tubules showed a reduction in weight to 37% at 7 months of age, but then an increase to 69% at 13 months of age as a sign of regeneration.

The striated ducts increased in weight to 140% at 7 months of age and to 155% at 13 months of age, and in accordance with the presumption for this calculation, there was no difference between shielded and unshielded glands (Fig 4).

DISCUSSION

Despite the low mitotic rate, human salivary gland tissue is radiosensitive and loses its function regularly after exposure to tumor therapeutic doses. However, the response and histologic changes after irradiation differ widely among different species and are dependent on dose and time intervals.

The rabbit is a common experimental animal, but the effects of radiation on rabbit submandibular glands have been only sparsely described. The weight loss after irradiation and its histologic background have been analyzed.[8,9] The proportions of the three major intralobular compartments of the glands were altered by irradiation and were dependent on the interval between radiation exposure and observation time. The size of the lobules was reduced, and extralobular fibrosis in the hilar region was increased. The seromucous acini were partly atrophied, with successively changed architecture and with reduced size of the granules. The serous tubules showed pronounced reduction of the granules at 4 months and a remarkable adenomatous regeneration at 10 months postirradiation. The striated ducts were almost unaffected during the observation time. Arteriolar changes were slight to moderate, and there was scarcely any capillary damage. The numbers of intralobular and extralobular plasma cells were increased (Fig 2B). Four and 10 months after irradiation, the mean weights of the unshielded submandibular glands of irradiated rabbits were 0.40 and 0.39 g, respectively — significantly less than the mean gland weight in the nonirradiated rabbit at the same ages, and significantly less than the corresponding values of the shielded glands of the same irradiated rabbits (0.76 and 0.87 g, respectively).[9]

The results obtained in this series of rabbits demonstrate that pilocarpine stimulation of salivary gland function during irradiation enhances the radiation damage as observed by macromorphological and micromorphological criteria in comparison with pharmacologically untreated rabbits.[9] In 2 cases a total atrophy of the seromucous acini and the serous tubules was seen, and the structures were replaced by fibrous tissue. This was never seen in rabbits irradiated with the same dose but without pilocarpine pretreatment.[9] Despite the more severe tissue injuries in these pilocarpine-treated animals, the striated ducts showed only swollen epithelium cells and regional widening of the lumina, but without any signs of damage.

The appearance of plasma cells was moderate to strong in pilocarpine-stimulated glands. The large number of remaining plasma cells at 10 months postirradiation indicated a still-active immunologic process, of which the functional importance is not known.

The effects of irradiation during pilocarpine treatment were also demonstrated by morphometric methods. The reductions in weight were in good correspondence with the histopathologic findings showing clear signs of severe radiation damage, and in some cases even total atrophy and fibrous replacement of the seromucous acini and the serous tubules (Fig 4). In comparison to corresponding nonirradiated control glands, the weight of both the shielded and unshielded pilocarpine-treated glands was reduced (Table 1). However, there was no significant histologic difference between the shielded glands of pilocarpine-treated irradiated rabbits and the control glands of nonirradiated rabbits. This may be explained by an inhibited gland growth in rabbits with the pilocarpine treatment that made them sensitive to the small dose of ≤2.7 Gy delivered to the shielded glands.

Abok et al[12] have reported that radiosensitivity of serous rat submandibular acinar cells increased with a higher content of secretory granules. Norberg and Forsberg[13] have shown that the α-adrenergic agonist cyclocytidine injected intraperitoneally in rats 1 hour before irradiation decreased the injury. The drug depleted the heavy metal granules of the serous cells of the submaxillary glands. However, the pharmacologically induced increase of gland function was insignificant during irradiation. This hypothesis is not contradictory to our findings that irradiation during, but not after, pilocarpine stimulation of the gland function produced more radiation damage.

Biperiden is a centrally active anticholinergic drug inhibiting rabbit salivary gland activity.[14] Rabbits treated with biperiden should consequently, on the average, have a somewhat lower salivation rate, which may have an effect on the radiation injury. The histologic examination revealed signs of injury of the same type as observed in rabbits without any pharmacologic treatment or treatment with pilocarpine prior to irradiation. However, the histologic signs of injury were generally less pronounced in comparison with pharmacologically untreated rabbits.[9] In the biperiden-treated glands, the seromucous acini had a much more normal appearance, there was only a moderate degranulation in the serous tubules, and the remaining granules had a normal appearance without any adenomalike structures. The fibrosis around the corresponding structures was less pronounced at both 4 and 10 months postirradiation, and the amount of plasma cells was only moderate at 10 months postirradiation.

The less severe effects of irradiation during biperiden treatment were clearly demonstrated also by histomorphometric methods. The weight of unshielded glands was higher in the group of biperiden-treated rabbits (0.66 and 0.63 g, respectively) compared to the group of nontreated rabbits (0.40 and 0.39 g,[9] respectively; Table 1). Inhibition of the submandibular gland function during irradiation seems to have a radioprotective effect.

It has been shown that administration of isoproterenol, amifostine (WR-2721), or cyclocytidine before irradiation will also lead to radioprotection.[12,13,15-18] However, the effect of irradiation on rabbit submandibular glands has not been reported.

Lotz et al[19] have shown that orciprenaline and carbachol administered before radiation protect salivary glands from injury. The effect was suggested to be explained by degranulation of secretory cells before irradiation. Degranulated cells have been shown to be less radiosensitive.[12,13,19] However, orciprenaline given repeatedly causes a hyperplasia of salivary gland parenchyma, and the effect can be explained by accelerated proliferation and repair of the injured tissues.[12,13,15-17,19] This mechanism can explain the radioprotective effect and is not contradictory to our observations.

The fact that radiosensitivity depends markedly on the oxygen present in the cell during irradiation may be relevant in these cases of pilocarpine or biperiden pretreatment. Our findings are in correspondence with the observation that an injection of vasopressin in the mesenteric artery, with inhibition of blood circulation and secretory function in the gut, will decrease the radiosensitivity of the enteric mucosa.[20] However, closer analysis demands measurements of intracellular PO_2 during pretreatment and irradiation.

Cherry and Glucksmann[21] claim that the patchy distribution of degenerative and regenerative phenomena, as well as of secretory activity within and between acini, cannot be explained by primary vascular or circulatory failure, but might be attributed to variations in the sensitivity of cells related to their functional state, ie, mitosis or secretory activity, or both. Our results support their opinion about the nature of radiation injuries in the salivary glands. The functional state of the parenchyma has an impact on the degree of the injury.

In conclusion, all histologic and morphometric criteria showed less pronounced radiation injury in glands irradiated during inhibition of the gland function, compared to glands irradiated during stimulation of the gland function, and also compared to glands without any pharmacologic treatment during irradiation. It was concluded that the functional status of the submandibular glands during irradiation probably has an impact on the later radiation injury.

This finding may offer a clinically important possibility of reducing salivary gland dysfunction after radiation treatment of tumors in the head and neck regions.

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CURRENT TRENDS, CONTROVERSIES, AND PERSPECTIVES IN RHINOLOGY AND SINUS SURGERY

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Ann Otol Rhinol Laryngol 103:1994

MYCOPLASMA AND CHLAMYDIA IN ADENOIDS AND TONSILS OF CHILDREN UNDERGOING ADENOIDECTOMY OR TONSILLECTOMY

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The prevalence of mycoplasmal and chlamydial infection was assessed in 83 children undergoing adenoidectomy, tonsillectomy, or both procedures for recurrent adenotonsillitis or obstructive symptoms. Throat smears (surface specimens) and minced adenoids and tonsils (core specimens) were cultured for *Mycoplasma* spp and for *Chlamydia* spp. Isolation rates in adenoidal specimens were as follows: *Mycoplasma hominis*, surface 7.1%, core 2.9%; and *Ureaplasma urealyticum*, surface 1.4%, core 2.9%. *Mycoplasma hominis* was also found in tonsillar specimens: surface 14.3%, core 20%. *Chlamydia trachomatis* was isolated only from a single core adenoidal specimen. The rate of mycoplasma isolation was significantly higher in children with recurrent adenotonsillitis (34.5%) than in those with obstructive symptoms (3.7%). Our findings document colonization of genital mycoplasmas in adenoids and tonsils of children with recurrent adenotonsillitis. Further studies are needed to evaluate the possible pathogenetic role of these microorganisms in adenotonsillar infection.

KEY WORDS — adenoidectomy, chlamydia, mycoplasma, tonsillectomy.

INTRODUCTION

The microbial flora of the tonsils and adenoids in children with recurrent adenotonsillitis and obstructive symptoms have been extensively investigated.[1-12] However, to the best of our knowledge, no study has dealt with chlamydial infection of the adenoids or tonsils, and only 1 study has dealt with mycoplasma adenotonsillar infection.[13]

In order to study the prevalence of mycoplasma and chlamydia infection in children with recurrent adenotonsillitis, we attempted to isolate these microorganisms from adenoids and tonsils removed at surgery.

MATERIALS AND METHODS

Patients. Children up to 15 years of age undergoing adenoidectomy and/or tonsillectomy for recurrent adenotonsillitis or obstructive symptoms of adenotonsillar hypertrophy (nocturnal dyspnea or snoring, impaired hearing) were enrolled consecutively in this study. For each patient, the following data were retrieved by interviewing the parents: medical history, recent symptoms, and antibiotic treatment (up to 1 month before surgery).

Mycoplasma and Chlamydia Cultures. Immediately before surgery, throat smears for *Mycoplasma* spp, *Chlamydia* spp, and classic aerobic bacteria were obtained with Transwab (MW 170, Medical Wire & Equipment Co, Potley, Corsham, England), Chlamydia Transwab (MW 935, Medical Wire & Equipment Co), and a sterile cotton swab (Transtube Sterile, Medical Wire & Equipment Co), respectively. These throat specimens (surface specimens), as well as the freshly excised adenoids and/or tonsils, were transported immediately to the microbiology laboratory. Following sterile dissection, the inner parts of the adenoids and tonsils (core specimens) were minced and cultured for *Mycoplasma* spp and *Chlamydia* spp. Mycoplasma surface and core specimens were inoculated into A7 broth and agar media (for *Ureaplasma urealyticum*), arginine broth and agar (for *Mycoplasma hominis*), and SP4 broth and agar (for *Mycoplasma pneumoniae*). Cultures on A7 and arginine media were incubated for 72 hours, and those on SP4 were incubated for 4 weeks. Cultures on solid media were incubated under strict anaerobic conditions. Mycoplasma identification was based on colony morphology and metabolic activities.[14] Chlamydia surface and core specimens were inoculated into cycloheximide-treated McCoy cells, centrifuged for 1 hour, and incubated for 48 and 72 hours, respectively. Cultures were stained with the iodine and Giemsa methods and examined for inclusion bodies. Classic aerobic bacteria were cultured and identified by standard methods.

Statistical Analysis. Contingency tables were tested for statistical significance by Fisher's exact test.

RESULTS

Eighty-three children were included in the study:

From the Department of Internal Medicine "C" (Huminer, Pitlik), the Department of Otolaryngology (Levy), and the Chlamydia and Mycoplasma National Reference Center, Department of Microbiology (Samra), Beilinson Medical Center, Petah Tiqva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

TABLE 1. CHARACTERISTICS OF 83 CHILDREN UNDERGOING OPERATION

	Adenoidectomy	*Tonsillectomy*	*Both*
No. of children	68	12	3
M:F ratio	1.4:1	2:1	2:1
Mean age (y)	5.2	6.2	4.8
Main respiratory symptoms			
Recurrent infections	20	8	1
Obstruction*	48	4	2

*Nocturnal dyspnea or snoring, impaired hearing.

TABLE 2. ISOLATION OF MYCOPLASMA AND CHLAMYDIA FROM SURFACE AND CORE SPECIMENS OF ADENOIDS AND TONSILS

Source of Isolation	Mycoplasma hominis	Ureaplasma urealyticum	Chlamydia trachomatis
Adenoids			
Surface	5/70 (7.1%)	1/70 (1.4%)	0/66
Core	2/68 (2.9%)	2/68 (2.9%)	1/52 (1.9%)
Tonsils			
Surface	2/14 (14.3%)	0/14	0/12
Core	3/15 (20%)	0/14	0/12

Data are number of isolates per number of available specimens.

68 of them underwent adenoidectomy, 12 tonsillectomy, and 3 both procedures. Their characteristics are presented in Table 1. While most subjects undergoing adenoidectomy suffered mainly from obstructive symptoms, most of the children undergoing tonsillectomy had mainly symptoms of recurrent infections. Thirty-three of the 83 children (39.8%) had received antibiotics during the month before the study as follows: amoxicillin 9 patients, co-trimoxazole 8, penicillin V potassium 7, erythromycin 4, cephalexin 1, and more than 1 antibiotic 4.

The results of mycoplasma and chlamydia cultures from surface and core specimens are presented in Table 2. Mycoplasmas were isolated from around 5% of the adenoids and from around 15% of the tonsils. Most of the isolates were identified as *M hominis*. *Ureaplasma urealyticum* was isolated only from the adenoids. *Mycoplasma pneumoniae* was not isolated from any of the specimens. *Chlamydia trachomatis* was isolated only from a single core adenoidal specimen.

In only 2 children did positive throat cultures for *Mycoplasma* spp predict isolation of these microorganisms from deeper tissues. Overall, mycoplasmas were isolated either from surface or core specimens of adenoids or tonsils of 12 children (14% of the study population). Table 3 presents the isolation rates of *Mycoplasma* spp in children with recurrent adenotonsillitis and in those with obstructive symptoms. While *M hominis* or *U urealyticum* was found in 34.5% of the children with recurrent adenotonsillitis, they were found in only 3.7% of those with obstructive symptoms ($p < .05$). *Mycoplasma* spp were isolated from 16% (5 of 32) of the children who had received antibiotics (other than erythromycin) prior to the surgery and from 15% (7 of 47) of those who had not received antibiotics (nonsignificant difference).

Throat cultures for classic aerobic bacteria were available in 60 children undergoing adenoidectomy and in 15 children undergoing tonsillectomy. In most cases (71.7% of children undergoing adenoidectomy and 75% of those undergoing tonsillectomy), normal "mixed" flora was found. Aerobic isolates included β-hemolytic streptococci (in 23.3% of the children undergoing adenoidectomy and in 12.5% of those undergoing tonsillectomy), *Streptococcus pneumoniae* (in 6.3% of subjects undergoing adenoidectomy and in none of those undergoing tonsillectomy), and *Staphylococcus aureus* (in 0.8% of children undergoing tonsillectomy and in none of those undergoing adenoidectomy). Sterile throat cultures were reported in only a few patients (3.3% of children undergoing adenoidectomy and 6.3% of those undergoing tonsillectomy).

DISCUSSION

Previous studies of the microbial flora in recurrent adenotonsillitis in children have demonstrated that mixed aerobic and anaerobic bacteria were present in the vast majority of patients.[1-12] The predominant aerobic isolates were α- and β-hemolytic streptococci, *S aureus*, pneumococcus, and *Hemophilus* spp.[2-4,7] Nonpathogenic *Neisseria* spp, *Corynebacterium* spp, *Branhamella catarrhalis*, *Eikenella corrodens*, *Moraxella* spp, *Escherichia coli*, and *Candida* spp have also been isolated.[6,8,10,11] β-Lactamase–producing organisms were frequently found.[2,3] In all studies that compared surface and core cultures, except 1,[9] there was a discrepancy between surface and core aerobic cultures.[1,2,11,12] A similar discrepancy has been detected regarding anaerobic bacteria.[2]

In only 1 study,[13] cultures for *Mycoplasma* spp were obtained and were positive in 54.5% of the patients. In that study *Mycoplasma* spp were cultured most consistently from the external surface of the adenoids. We could not find any previous study aimed at isolating *Chlamydia* spp from tonsils and adenoids. However, in several reports these microorganisms were detected in throats of persons with chronic otitis media or acute pharyngitis. Using a fluorescent-labeled monoclonal antibody, Banks et al[15] found that 44% of children with chronic otitis media had positive throat smears for *C trachomatis*.

TABLE 3. ISOLATION RATES OF *MYCOPLASMA* SPP IN CHILDREN WITH RECURRENT ADENOTONSILLITIS AND IN CHILDREN WITH OBSTRUCTIVE SYMPTOMS

Clinical Presentation	*Patients Tested*	*Patients With Positive Mycoplasma Cultures*
Recurrent adenotonsillitis	29	10 (34.5%)
Obstructive symptoms	54	2 (3.7%)*

*$p < .05$.

Recently, *Chlamydia* sp strain TWAR (*Chlamydia pneumoniae*) and *M pneumoniae* were isolated from the throats of 8% and 9%, respectively, of adults with acute pharyngitis.[16] In none of these patients could *C trachomatis* be isolated. Komaroff et al[17] found serologic evidence of infection with *C trachomatis* and with *M pneumoniae* in 20.5% and 10.6%, respectively, of 763 adults suffering from acute pharyngitis. However, other investigators[18-20] failed to isolate *C trachomatis* from pharyngeal cultures of adults and children with acute pharyngitis.

In our study, a relatively low prevalence of aerobic bacterial infections was found. It can be attributed to recent antibiotic treatment in many of the children. Contrary to the single previous study reporting a high prevalence of mycoplasmal infection of tonsils and adenoids,[13] we isolated *Mycoplasma* spp (*M hominis* or *U urealyticum*) in less than 8% of the adenoids and in no more than 20% of the tonsils. Pretreatment with erythromycin or other antimycoplasmal antibiotics could explain negative cultures only in a few cases. The similar percentage of mycoplasma recovery from children who had received antibiotics prior to the surgery and from those who had not received antibiotics stands against the possibility that prior antibiotic therapy had selected for or eradicated the study organisms.

Isolation of *Mycoplasma* spp in our study was more prevalent from the tonsils than from the adenoids. Similar to the study by Sprinkle et al,[13] surface cultures performed by us did not reliably predict the presence of mycoplasma in the core specimens.

The prevalence of *Chlamydia* spp isolation was very low in the present study. In only 1 of the patients was *C trachomatis* isolated, and *Chlamydia psittaci* was not found in any of the children. However, we did not perform specific cultures for *C pneumoniae*, which might have been found more frequently in the study population.

Most mycoplasma isolates were identified as *M hominis*. This species is uncommon in extragenital infections.[21] In previous studies, it has been isolated from up to 6% of persons with acute pharyngitis or acute upper respiratory tract illness[22] and has been found to induce exudative pharyngitis in susceptible volunteers.[23] However, it has not been implicated definitely in the causation of these infections.[22]

Ureaplasma urealyticum has been found to colonize about 40% of the throats of neonates born to mothers with positive vaginal cultures for this microorganism.[24] The colonization rates of these genital mycoplasmas were found to decline steadily toward zero during the first year of life.[24] The results of our study suggest that upper respiratory colonization with genital mycoplasmas may be more common in children than previously thought. Our findings of higher rates of mycoplasma colonization in children with recurrent adenotonsillitis (34.5%) as opposed to those with obstructive symptoms (3.7%) may suggest that mycoplasmas could be involved in the pathogenesis of adenotonsillitis. However, recommendation of mycoplasma culture in evaluation of children with recurrent adenotonsillitis may be premature, in view of the lack of information on colonization rates in a control group and the uncertainty about the pathogenic role of mycoplasma in adenotonsillitis. Further investigation is needed to evaluate these unanswered questions.

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CHANGES IN GLOTTAL AREA ASSOCIATED WITH INCREASING AIRFLOW

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Laryngeal resistance varies inversely with airflow during phonation. This study evaluated the morphological changes in the glottis that accompany decreases in laryngeal resistance at higher levels of airflow. An in vivo canine model of phonation and a video analysis system were used to assess changes in area. Four animals were examined stroboscopically as airflow increased, with constant recurrent laryngeal nerve stimulation. Glottal dynamics were evaluated by means of photoglottography, electroglottography, and measures of subglottic pressure. Analysis of digitized stroboscopic images indicated that increasing airflow had no obvious effect on the glottal chink (vocal process contact). Increasing airflow was associated with an increase in the area of peak opening and an increase in the glottal area integral.

KEY WORDS — airflow, glottal area, laryngeal resistance, larynx, phonation.

INTRODUCTION

Laryngeal resistance is defined as mean subglottic pressure divided by mean airflow rate.[1] The decrease in laryngeal resistance occurring at higher airflow has two possible explanations. First, the duration or frequency of laryngeal opening may increase with increasing airflow. Alternatively, examination of serial videostroboscopic images suggests that the decrease in laryngeal resistance may be due to increases in glottal area with increasing flow. Analysis of electroglottography (EGG) waveforms has shown that the open quotient (defined as the duration of glottal opening divided by the duration of the cycle) increases with airflow.[2] Glottal area at various levels of airflow has not been measured directly.

The present study uses techniques developed recently in our laboratory to quantify morphological features of the vibrating larynx, including glottal area, from videostroboscopic images.[3] Glottal area integral over one cycle of phonation can be calculated from these measures by synchronizing the videostroboscopic images with EGG waveforms and then plotting area versus time. These techniques allow measurement of the effect of changes in airflow on the morphology of the vibrating larynx. Without such in vivo measurements, the relationship between glottal geometry and airflow has been limited to theoretic discussion.

MATERIALS AND METHODS

In Vivo Canine Model. The in vivo canine model of phonation is depicted in Fig 1, and has been described in detail in previous reports.[4] Four mongrel dogs (approximately 20 kg each) were premedicated with acepromazine maleate intramuscularly. Intravenous thiopental sodium was administered to a level of corneal anesthesia. Additional thiopental sodium was

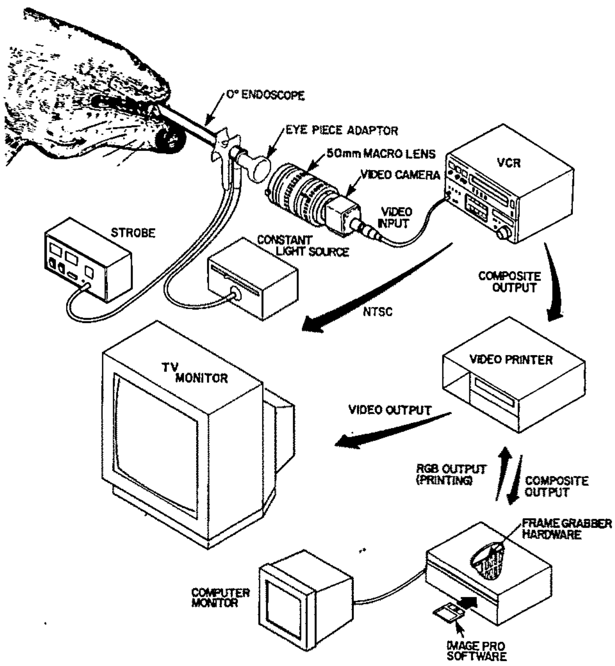

Fig 1. System used for measurement of videostroboscopic images in in vivo canine model of phonation. NTSC — National Television Standards Commission, RGB — red, green, blue.

From the Division of Head and Neck Surgery, University of California, Los Angeles, California. Supported by the American Laryngological Association Research Award. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Los Angeles.

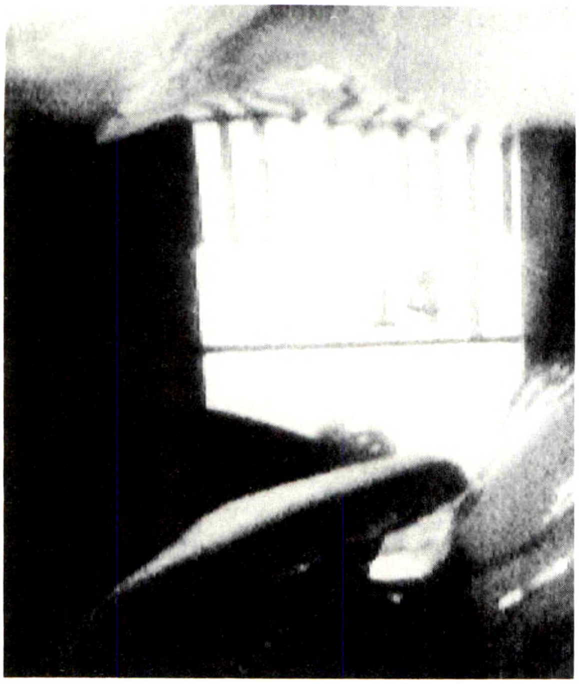

Fig 2. Centimeter ruler lowered to level of glottis and measured in pixels to allow conversion from pixels to square millimeters.

used to maintain this level of anesthesia throughout the procedure.

Animals were placed supine on the operating table. A midline incision was made to expose the trachea from the hyoid bone to the sternal notch. A low tracheostomy was performed at the level of the suprasternal notch and cannulated with an endotracheal tube for ventilation. A second tracheostomy was performed superiorly and a cuffed endotracheal tube was passed in a rostral direction with the tip positioned approximately 10 cm below the vocal folds. The cuff was inflated and air was passed through this rostral endotracheal tube from a laboratory wall outlet. The airflow was humidified and heated by bubbling it through 5 cm of heated water so that the temperature of the air was 37°C when measured at the glottal outlet.

Upstream subglottic pressure was measured with a catheter-tipped pressure transducer (model SPC-330; Millar Instruments, Houston, Tex). The subglottic pressure transducer was passed rostrally through the superior tracheostomy and placed 1 cm below the glottis.

Airflow was controlled by a valve at the laboratory wall outlet and measured with a flowmeter (model F1500; Gilmont Instruments, Great Neck, NY). One-centimeter segments of recurrent (RLN) and superior (SLN) laryngeal nerves were isolated and Harvard bipolar electrodes (South Natick, Mass) were applied. A constant current nerve stimulator (model S2LH; WR Medical Electronics Co, St Paul, Minn) was used to stimulate the RLNs, and a constant voltage source (model 54H; Grass Instruments, Quincy, Mass), was used to stimulate the SLNs. These nerves were stimulated at 70 to 80 Hz, with 0.3 to 0.5 mA (RLN) or 1 to 1.4 V (SLN) intensity for 1.5 milliseconds' pulse duration. Phonation was produced by discrete airflow intervals from 100 mL/s to 550 mL/s.

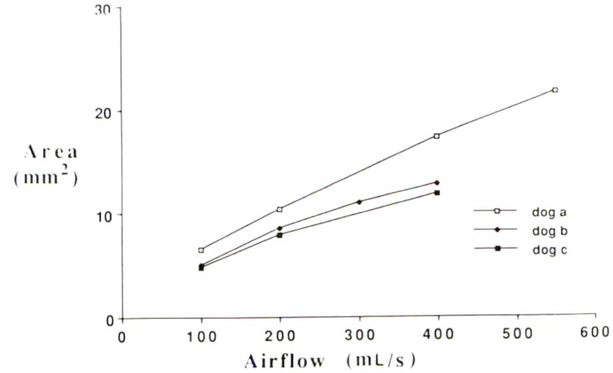

Fig 3. Peak glottal area versus airflow.

The photoglottography (PGG) and subglottic pressure signals were low-pass-filtered at 3 kHz, digitized at 20 kHz for 2.8 seconds, and stored on the hard disk of a personal computer.

Stroboscopy. Stroboscopic videoendoscopy was performed with a Karl Storz research stroboscope (model 8000, Culver City, Calif) used to illuminate the glottis through a 0° documentation sheath. Images were recorded with a charge-coupled device camera (model 70-5110; Jedmed, St Louis, Mo) and a 3/4-in professional videocassette recorder (model VO9850; Sony, Park Ridge, NJ).

Synchronization. The synchronization method used is described by Berke et al.[5] A custom-made Kluge box produced a 5-millisecond square wave pulse (SWP). A Hitachi oscilloscope (model V-1050 F; Torrance, Calif) was used to separate the vertical synchronization trace of the video signal. Berke et al[5] showed previously that a SWP can be digitized and simultaneously recorded on the sound track of a videotape. A strobe flash is simultaneously recorded onto the PGG waveform, where it produces a sharp increase in the PGG signal. By recording both the SWP and the strobe flash, the timing of any stroboscopic image can be determined. By referring to the EGG waveform, the amount of time between the opening of the glottis and the recording of a given image can also be calculated.

Digitization. A Labmaster analog-to-digital microprocessor board housed in a personal computer

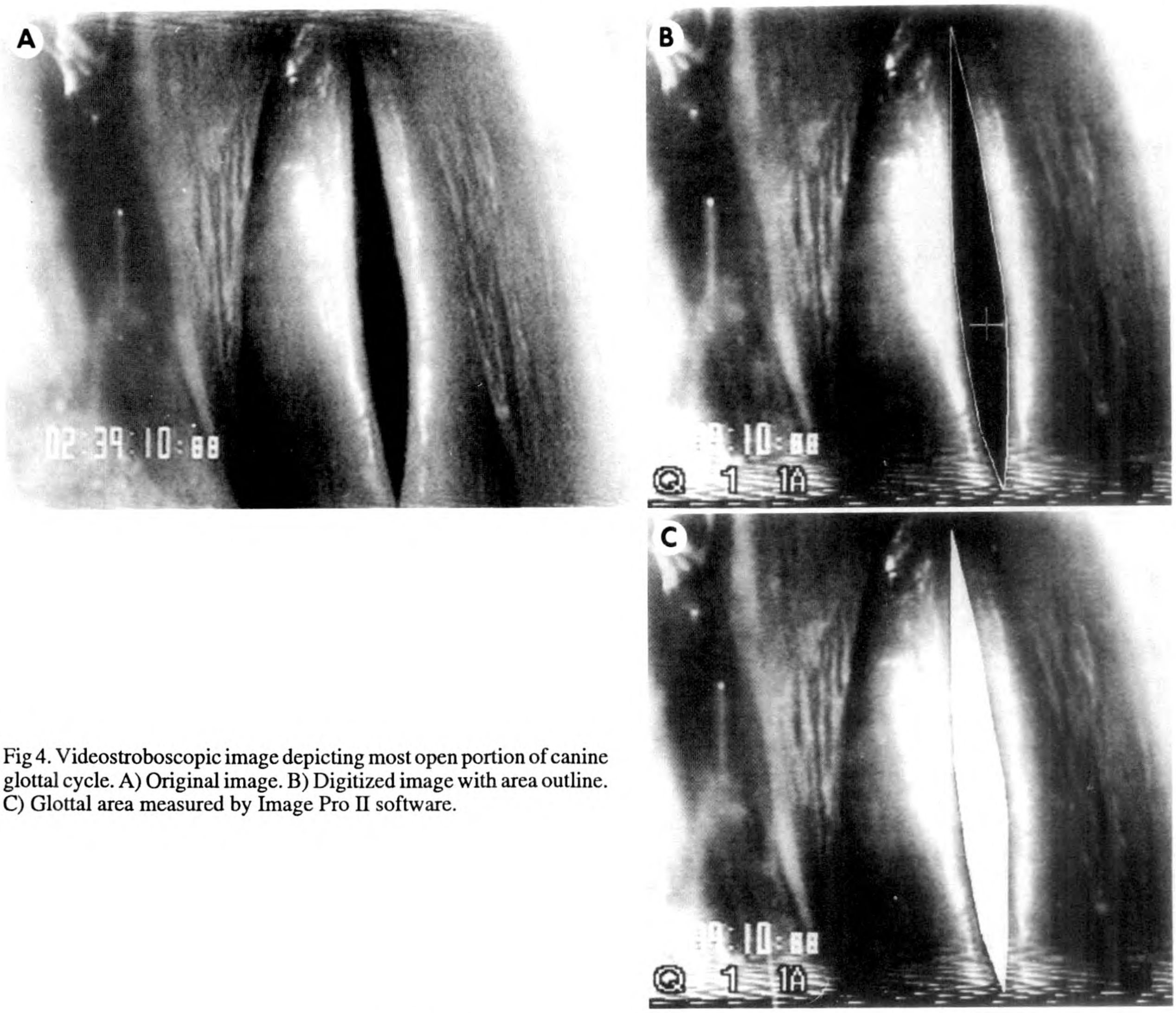

Fig 4. Videostroboscopic image depicting most open portion of canine glottal cycle. A) Original image. B) Digitized image with area outline. C) Glottal area measured by Image Pro II software.

with a math coprocessor allowed for four channels to be recorded: 1) EGG waveforms, 2) PGG waveforms, 3) SWP signals, and 4) the vertical trace of the video signals. The EGG and PGG signals were verified on a Tektronix oscilloscope (model 5116; Beaverton, Ore) before recording.

Videostroboscopic Image Evaluation. Glottal opening (area) was measured with a mouse-driven software package (Image Pro II, Media Cybernetics; Silver Spring, Md). The hardware necessary for area measurements in the dog is shown in Fig 1. The videostroboscopic image was first digitized by a Frame Grabber board (Data Translation, DT-2853 60SQ; Marlborough, Mass). After outlining of the desired portion of the digitized image, the area of the measured trace was expressed in pixel units by the software.

Figure 2 illustrates the calibration method used for an in vivo canine experiment. A standard centimeter ruler was lowered to the level of the glottis and measured with the software, so that pixel units could be converted to square millimeters. The camera was kept at a constant distance from the larynx throughout each experiment.

Experimental Design. Three animals were studied by varying airflow from 100 to 550 mL/s at three to four discrete levels of flow. The peak area of each complete glottal cycle was calculated from 8 to 12 stroboscopic cycles. Each stroboscopic cycle lasted approximately 1 second and therefore represented several hundred cycles of vibration, depending on the frequency of phonation. The measures for each animal at each flow rate were averaged and the results plotted. The laryngeal nerve stimulation (RLN and SLN) was kept constant during the trials for each animal. To ensure that fatigue was not altering the results, the subglottic pressure was periodically assessed at a median flow rate (318 mL/s) and found to be constant.

One additional animal was studied to determine

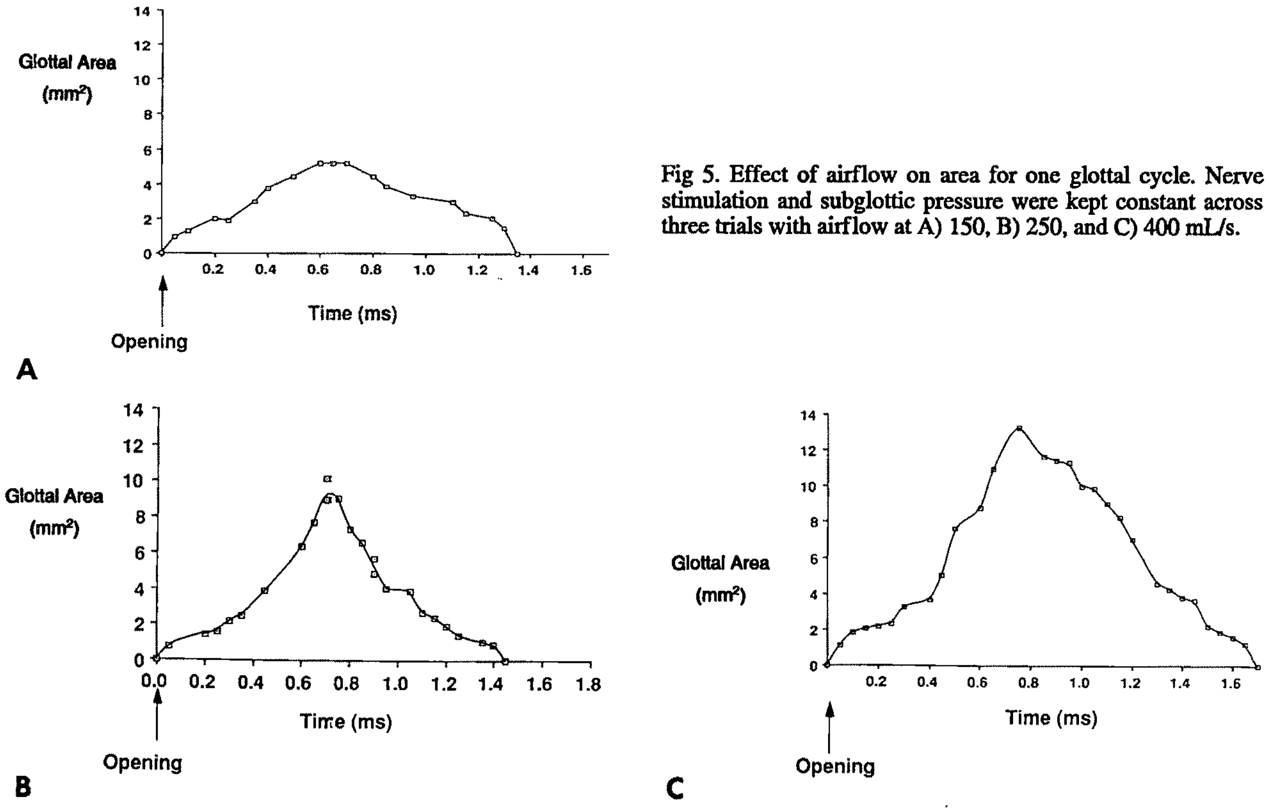

Fig 5. Effect of airflow on area for one glottal cycle. Nerve stimulation and subglottic pressure were kept constant across three trials with airflow at A) 150, B) 250, and C) 400 mL/s.

the integral of glottal area at three levels of flow. To determine the area for each point during the glottal cycle, the area of a series of images from one stroboscopic "cycle" was measured. The area was measured from each image and a graph relating area versus time was generated.

RESULTS

Because of individual variation in the simulated phonation in the animal subjects, peak area could not be measured at every level of flow for every dog. Consequently, dogs could not be combined in a single statistical analysis. However, a one-way analysis of variance (ANOVA) showed no significant overall differences among dogs in peak glottal areas across flow levels ($F_{2,8} = 1.400$, $p > .05$). Dogs were therefore combined for the following analysis.

Simple linear regression showed a significant and substantial relationship between airflow and peak glottal area ($F_{1,9} = 71.36$, $p < .01$; $r^2 = .89$). This relationship is shown in Fig 3.

DATA ANALYSIS OF FIGURE 5

Figure	Airflow (mL/s)	Peak Subglottic Pressure (mm Hg)	Area Integral (mm²/s)	R^2	Frequency (Hz)
5A	150	24.9	3.99	.98	251
5B	250	23.8	5.48	.901	268
5C	400	24.9	10.19	.935	287

Fifth-order polynomial was generated for each curve and integrated to calculate area integral. R^2 is correlation coefficient of curve fit.

A sample videostroboscopic image of peak opening is shown in Fig 4A. Figure 4B,C shows the same image after digitization by the Frame Grabber hardware and measurement by the Image Pro software.

Figure 5 shows plots of the glottal area versus time waveforms from sequential glottographic images at three different airflow rates (150, 250, and 400 mL/s) in the same animal. Each area point represents one image from consecutive stroboscopic cycles. Time zero corresponds to the moment of opening of the upper vocal fold margins. The last point on each

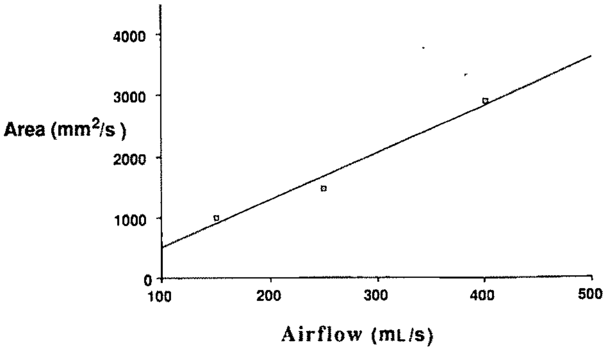

Fig 6. Product of area integral and fundamental frequency, versus three airflow rates (150, 250, and 400 mL/s). This measurement approximates "area per second."

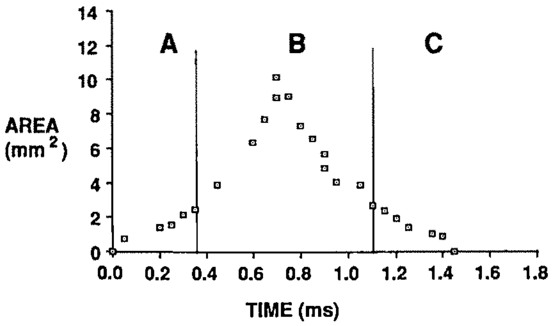

Fig 7. Events (laryngeal cycle) observed videostroboscopically corresponding to three portions of area waveform. A — upper margin unzipping, B — transverse displacement, C — "rezipping" (lower margin). See text for discussion.

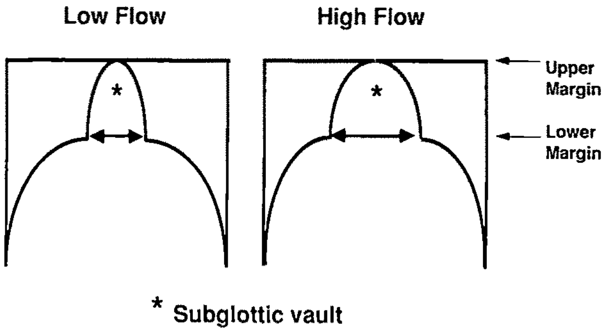

Fig 8. Proposed effect of airflow on subglottic vault. Higher airflow dilates subglottic vault, leading to greater area of opening.

curve corresponds to the instant of closure of the lower folds. The timing of each image relative to laryngeal opening was determined by synchronizing the image to glottographic and subglottic pressure signals. A best-fit fifth-order polynomial was obtained for each glottal waveform by means of a commercial graphing software package (Cricket Graph; Garden City, NY). The mathematic equation was integrated to determine the area under the glottal area versus time waveform for one glottal cycle.

The glottal area integral, representing the cumulative glottal opening (area) during one glottal cycle, is outlined in the Table. Laryngeal nerve stimulation was kept constant during this experiment as the flow rate was altered. The integral was multiplied by the fundamental frequency in hertz to determine the glottal area per second of phonation in the model. This was plotted against airflow for three different flow rates in the same animal (Fig 6). This product increases with increasing airflow in a nearly linear fashion, although statistical analysis was not attempted because of the very small number of data points.

Analysis of the videostroboscopic data revealed no posterior glottal gap. In addition, no difference in interarytenoid distance was observed following changes in airflow.

DISCUSSION

Glottal area has previously been estimated by PGG, a measure of light passing through the glottal opening, and EGG, a measure of electrical impedance across the vocal folds. Quantitative measurements of glottal area have been made with high-speed photography, which is expensive and labor-intensive.[6] This study provides direct measurements of glottal area through use of stroboscopic data and image analysis software.

The integral of the glottal area versus time waveform is the most meaningful method of examining the effect of airflow on glottal geometry. The glottal area integral evaluates both the duration and magnitude of opening, while an open quotient measures only the duration of opening, and the peak area assesses only the magnitude of glottal opening. Multiplying the area integral by the fundamental frequency provides a measure of the glottal area per second. This product increased with airflow in a nearly linear fashion (Fig 6). This relationship explains the decreases in laryngeal resistance that occur with increasing airflow.

The shapes of the glottal area versus time waveforms can be understood by analyzing the videostroboscopic sequences. In the first portion of the cycle (labeled A in Fig 7), glottal area increases slowly during unzipping of the upper margin of the vocal fold. During the middle portion of the cycle (labeled B), lateral displacement of the vocal cord occurs. This results in an abrupt increase in area. Finally, the lower margin of the vocal fold "rezips," causing flattening of the curve.

Peak area increased significantly with increased flow in the in vivo canine model. These findings are consistent with the canine study of Smith et al,[2] who analyzed glottal area from video screen tracings. Figure 8 shows one possible explanation for this finding. At higher airflow, a greater dilatation of the subglottic vault occurs before vocal fold opening. This increase in subglottic vault translates to increased excursion amplitude during vibration.

In these experiments increased airflow at the same level of nerve stimulation resulted in an increased area and therefore a decrease in glottal resistance. These conclusions were also reached by Smith et al[2] in 1991 and are consistent with Ohm's law for fluid systems. Figure 7 presents a possible explanation for the increase in area that occurs at greater airflow levels. The Figure shows the glottis at the moment before opening of the upper margins. With higher airflow, a greater dilatation of the subglottic vault

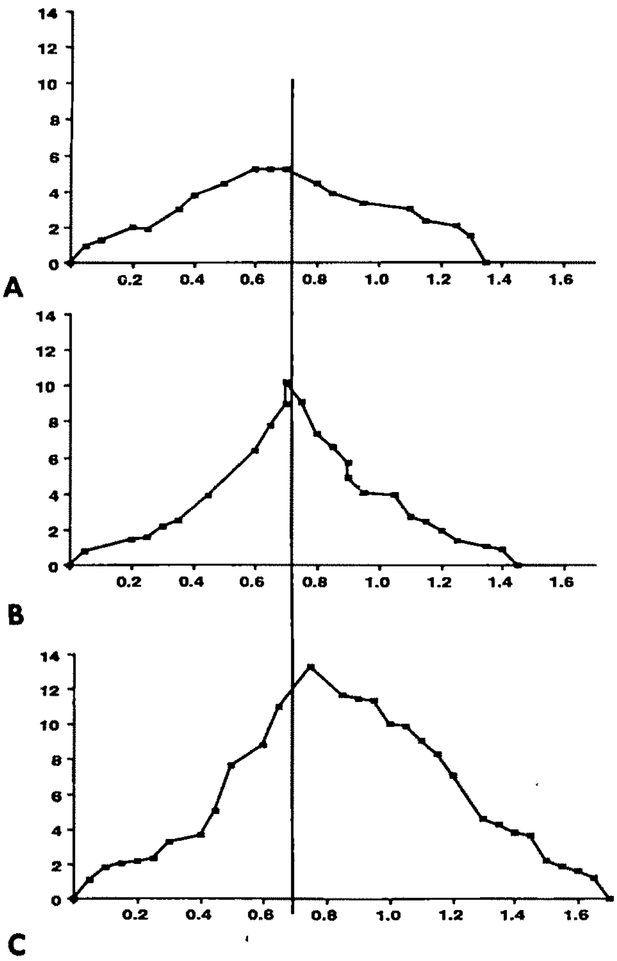

Fig 9. Timing (x-axis; in milliseconds) of peak glottal opening (y-axis; area in square millimeters) at three levels of airflow: A) 150, B) 250, and C) 400 mL/s.

exists at this point in the cycle. This hypothesis is supported by the collapsible tube model of phonation as described by Berke et al.[7] At low flow rates, a higher resistance system exists when the subglottis and glottis are collapsed, while high flow results in a distended glottis and a lower resistance to airflow. The changes in the area of the subglottic vault are seen in the regions of the negative pressure differential versus flow of the collapsible tube model.

The findings in this experiment contradicted those of Tully et al,[8] who studied the relationship between thyroarytenoid activity and laryngeal resistance. They reported no increase in "glottal aperture" with increasing flow. It is not clear how the glottal aperture was assessed in that experiment, and therefore it is difficult to determine the cause of the discrepancy.

Another part of the explanation of the increased glottal area integral with increasing airflow is provided by Fant,[9] who hypothesized that relatively constant subglottic pressure should produce constant exit jet velocity despite increasing airflow. From Ohm's law of flow it follows that $Q = A \times f \times V$, where flow through the glottis (Q) is equal to the product of the area integral of the glottis (A), the frequency of vocal fold vibration (f), and the velocity of flow (V). Therefore, if flow increases and exit jet velocity is constant, the area integral increases.

Also, a careful analysis of the glottal area waveforms suggested that the peak glottal area occurs at a similar time relative to the initiation of glottal opening, regardless of the absolute value of airflow (Fig 9). The enlarged subglottic vault imparts more energy for opening the vocal folds, not only with a greater peak area, but also with greater displacement velocity.

In the future, our laboratory plans to extend this work by measuring the effect of increasing laryngeal resistance on glottal area by altering laryngeal nerve stimulation at constant airflow rates.

In conclusion, glottal area increased with increasing airflow, both when it was measured as peak area and when an integral was calculated over the area versus time curve. This study demonstrates the feasibility and power of recently developed techniques for the objective study of laryngeal vibration.

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FUNDAMENTAL FREQUENCY AND AMPLITUDE PERTURBATION IN RECONSTRUCTED CANINE VOCAL FOLDS

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A submucosal fat autograft was implanted within the cover of injured vocal folds of 5 dogs. The implant occurred 6 weeks after unilateral mucosal excision had been performed. Three months postoperatively the larynges of these animals were excised and their phonation was compared to that of normal dog larynges and to other larynges with mucosal excision (but without fat grafting). Radiated acoustic pressure from the artificially driven larynges was recorded and digitized at 20 kHz with 16-bit resolution. Amplitude and fundamental frequency perturbations were extracted from a segment of phonation to assess the stability of the acoustic signals from the 3 groups. It was found that fat augmentation after mucosal excision reduced amplitude and frequency perturbation measures. There was no significant difference between fat-augmented and normal vocal folds. The acoustic measures were also positively correlated with phonation threshold and phonation efficiency measures reported earlier. The results suggest that submucosal fat autograft implantation within an injured vocal fold cover can restore not only the "ease" of phonation, but also the stability of phonation, which is a component of vocal quality.

KEY WORDS — acoustic measures, amplitude perturbation, canine vocal fold, fat autograft, fundamental frequency, vocal fold augmentation, vocal fold reconstruction.

INTRODUCTION

This paper is a follow-up to an earlier study by the same authors[1] on a phonosurgical procedure involving a submucosal autograft of fat in an injured canine vocal fold. The purpose of the earlier study was to investigate if a functional lamina propria could be maintained by grafting. After the operation and an extended recovery period, the larynges were harvested and phonated on a laboratory bench. It was found that the fat-augmented vocal folds had lower phonation threshold pressure, greater vocal intensity, and greater glottal efficiency than the injured vocal folds without fat grafting. These results suggested that this phonosurgical procedure improves laryngeal function, at least as far as measures of "ease" of phonation are concerned.

Normal vocal function is not represented by "ease" of phonation alone, but also by stability of phonation. It was deemed appropriate, therefore, to follow up with some perturbation measures. A series of publications have appeared that relate specific or general laryngeal diseases, and the perception of vocal disorders, to a variety of perturbation measures.[2-7] It seems that jitter and shimmer increase and the signal to noise ratio decreases in most pathologic cases.

Phonatory stability is sensitive to structural characteristics of the vocal folds. Scarring of vocal fold tissue can result in irregular vibration, which can manifest itself as hoarseness or loss of sonority. A series of studies by Hirano and Kakita[8] and more recent theoretic work by Titze et al[9] have supported a body-cover theory of vocal fold vibration. In this theory, the vocal fold is represented as a layered structure having a cover, which consists of the surface epithelium and the underlying superficial lamina propria, and a body, consisting of the deeper lamina propria and the vocalis muscle. A mathematic analysis of the body-cover model shows that the compliant nature of the lamina propria is critical to normal vocal fold vibration.[9] Further physiologic significance of the cover in phonation is discussed by Cooper.[10] Animal studies have demonstrated that loss of the superficial layer and chronic fibrotic changes in the lamina propria occur after vocal fold stripping.[11] Therefore, phonosurgical procedures should be conducted with maximum prevention of scarring and maximum restoration of a functional lamina propria.

The purpose of this study was to use acoustic

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REPRINTS — Ingo R. Titze, PhD, Dept of Speech Pathology and Audiology, The University of Iowa, Iowa City, IA 52242.

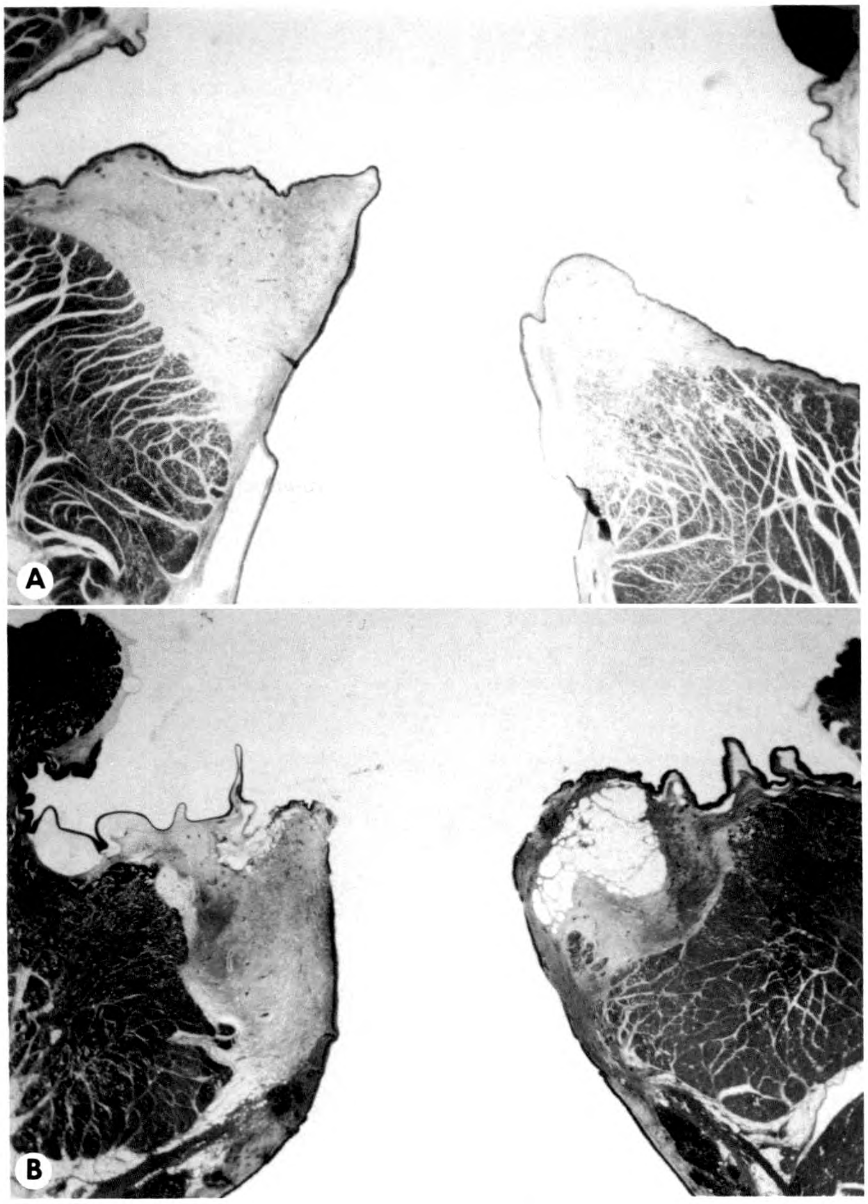

Fig 1. Coronal sections. A) Through normal vocal fold (left side) and reepithelialized vocal fold after mucosal excision (right). B) Through normal vocal fold (left) and fat-grafted vocal fold (right).

perturbation analysis to investigate the effects of a vocal fold augmentation procedure on the stability of phonation in scarred canine vocal folds. It was our hypothesis that if phonation stability could be restored in these damaged vocal folds, along with the intensity and glottal efficiency described earlier,[1] then the quality of the sound would be improved along with the "ease" of phonation.

METHOD

Thirteen female dogs weighing 15 to 20 kg were used in the earlier study. Four animals served as the unoperated group, hereinafter referred to as the normal group. The other 9 animals underwent a right true vocal fold mucosal excision endoscopically. The excisions were directed along the leading vocal fold edge, at a depth to include the upper to middle lamina propria. After the mucosal excision, 4 of the 9 animals had no further operative intervention. They are referred to as the mucosal excision group.

After 6 to 8 weeks of healing from the mucosal excision, the remaining 5 of the 9 mucosal excision animals underwent a right vocal fold fat autograft augmentation. This group will be referred to as the fat graft group. Through a midline laryngofissure approach, a submucosal fat autograft was implanted within the injured vocal fold cover.

Coronal section photomicrographs were taken of the vocal folds. In the normal group, the lamina propria was well developed (Fig 1, left side of A and B). In the mucosal excision group, reepithelialization occurred, but a deficiency of the lamina propria thickness remained apparent 2 months after excision

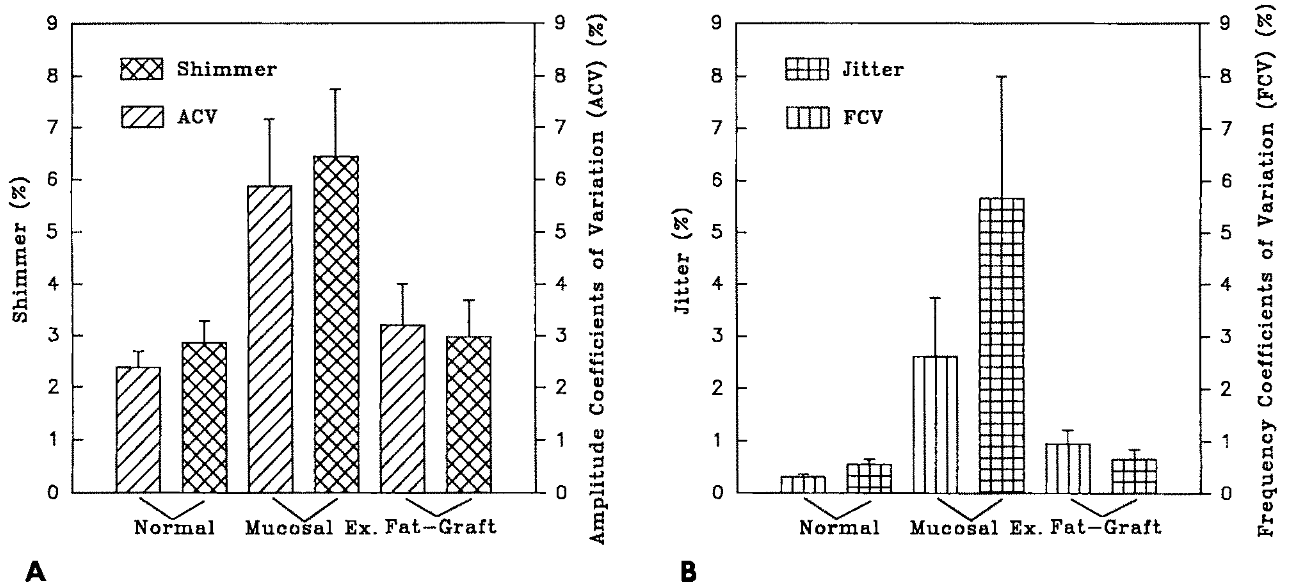

Fig 2. A) Amplitude coefficient of variation (ACV) and shimmer and B) fundamental frequency coefficient of variation (FCV) and jitter obtained from acoustic radiated pressure for 3 experimental groups. Ex. — excision.

(Fig 1A, right side). In the fat graft group, the implant remained localized near the leading edge of the vocal fold after 2 months (Fig 1B, right side).

All larynges of the 3 groups of animals were harvested 3 months postoperatively. The performance of the larynges was studied with an excised larynx setup.[12,13] A humidified air column with regulated flow provided a subglottic airstream while the subglottal pressure was monitored. Radiated acoustic pressure was recorded on the audio channel of a Panasonic video cassette recorder and digitized at 20 kHz with 16-bit resolution (DSC 240 analog-to-digital converter) on a VAX station 3200. Four tokens of phonation were used for each larynx. Each token was 1 second in duration. A waveform matching algorithm[14,15] was used to evaluate the frequency and amplitude stability (jitter and shimmer) of every token for all 3 groups.

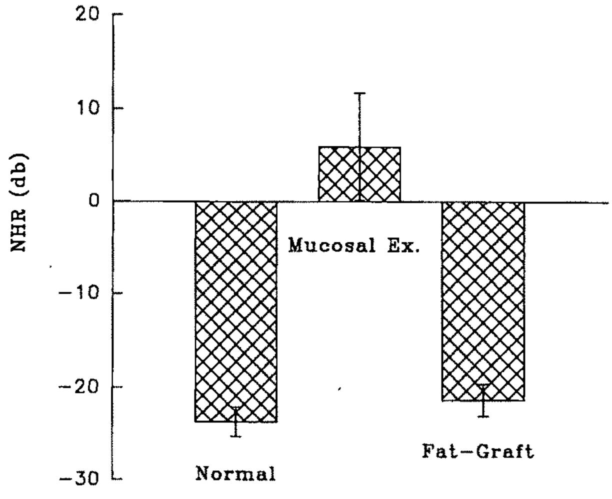

Fig 3. Noise to harmonic ratio (NHR) obtained from acoustic radiated pressure for 3 experimental groups. Ex. — excision.

RESULTS

Figure 2A shows shimmer and amplitude coefficient of variation (ACV; standard deviation divided by the mean) of the microphone signal for each group. Shimmer was computed as the mean rectified adjacent-cycle difference between peak-to-peak amplitudes, whereas ACV was computed as the root mean squared difference from the mean.[16] Comparatively, shimmer relates more to the short-term fluctuation in amplitude, whereas the ACV relates more to long-term fluctuations. Error bars indicate the standard error of the mean for each measure (0.05 for shimmer and 0.06 for ACV). It is seen that the fat-augmented vocal fold group had significantly lower amplitude perturbation measures than the mucosal excision group and was essentially equivalent to the normal group.

Figure 2B shows jitter and the coefficient of variation of fundamental frequency (FCV). Again, the fat-augmented vocal folds had significantly lower values than the mucosal excision group. Values for both normal and fat graft groups were less than 1%, which is typical for normal human steady phonation.[4,17] A third measure, the noise to harmonics ratio (which is the inverse of the harmonics to noise ratio described by Yumoto et al[5]), is shown in Fig 3. This shows most dramatically the difference in regularity of vibration between the mucosal excision group and the 2 other groups. An approximate –20-dB noise to harmonics ratio (or +20-dB harmonics to noise ratio) is typical for human phonation. The mucosal excision group had about 25 to 30 dB more noise energy in the acoustic signal than either the normal group or the fat graft group.

DISCUSSION AND CONCLUSION

As an addendum to results reported in an earlier study,[1] the present results suggest that submucosal fat autograft implantation in an injured vocal fold cover can restore the stability (periodicity) of the acoustic output. This is in addition to the improvement in "ease" of phonation described earlier in terms of vocal efficiency and phonation threshold pressure. Because acoustic stability is a component of vocal quality, the results indicate that the fat autograft may improve overall phonation quality of a damaged vocal fold, although no formal quality judgments were made.

Generally, the purpose of reconstructive procedures is to make the vocal folds more symmetric. Restoration of symmetry should include both geometric and viscoelastic properties of the vocal folds. It appears that a fat graft can offer some improvement in both tissue properties and vocal fold shape.

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DISTRIBUTION OF CYTOKERATINS IN THE VESTIBULAR EPITHELIUM OF THE GUINEA PIG

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Cytokeratin expression in the vestibular labyrinth of the guinea pig was investigated with immunofluorescence and immunoperoxidase staining on surface preparations of the vestibular epithelium. Phalloidin, an F-actin–specific probe, was used to distinguish between hair cells and supporting cells. Cytokeratin expression was not found in the cytoplasmic domain of hair cells of the crista ampullaris, utricle, or saccule. Cytokeratin expression was abundant in supporting cells of the vestibular sensory epithelium. Electron microscopy revealed the presence of desmosomes, which are associated with cytokeratins, within type 2 hair cells of the vestibular epithelium. It appears that cytokeratins are absent within the cytoplasmic domain of hair cells, but are present in association with intercellular junctions. The functional significance of this unique pattern of cytokeratin expression within the vestibular epithelium is discussed.

KEY WORDS — actin, cytokeratin, guinea pig, hair cell, organ of Corti, vestibule.

INTRODUCTION

The cytoskeleton of most cells comprises three main components: microfilaments, intermediate filaments, and microtubules. Microfilaments are fibrils that are 5 to 7 nm in size, intermediate filaments are those that are 10 nm in size, and microtubules are 25 nm in size. Intermediate filaments are classified into five groups according to their tissue-specific expression: cyto-keratin, vimentin, neurofilament, desmin, and glial fibrillary acid protein.[1] Cytokeratins are unique to epithelium-derived cells. Vimentin is expressed by mesenchyme-derived cells. Neurofilaments are found in neuronal cells. Desmin is expressed by cells of myogenic origin, and glial fibrillary acid protein is typical of astrocytes. These five different classes of intermediate filaments and subsets can be used to identify a cell's embryonic origin.[2] Because the expression of intermediate filaments is maintained in differentiation and tumor formation, a malignant cell's tissue of origin can be determined.[2] During embryonic development, different intermediate filaments and combinations of intermediate filaments may be expressed by the same cell.[1] Hence, the developmental stage of a cell can be determined by its intermediate filament expression. Postnatal coexpression of different intermediate filaments within the same cell has been documented in some cell types.[3,4] Cytokeratins and intermediate filaments in general are the most stable components of the cytoskeleton and help maintain cellular organelle position.[1,5]

Recently, the laminins were added as a separate class of intermediate filaments.[6,7] On the basis of their biochemical structure, the nuclear laminins and five classes of cytoplasmic intermediate filaments can be divided into five sequence types.[6,7] Sequence typing of intermediate filaments categorizes together those intermediate filaments that have identical central rod domains, based on protein primary sequence comparisons. Sequence type 1 comprises the acidic keratins. Sequence type 2 comprises the basic or neutral keratins. Sequence type 3 includes desmin, vimentin, and glial fibrillary acidic protein. Sequence type 4 comprises the neurofilaments, and sequence type 5, the laminins. Both acidic and basic cytokeratins are coexpressed by epithelial tissues. Cytokeratins are a group of intracellular α-fibrous proteins associated in a filamentous form with an approximate diameter of 10 nm. Cytokeratin filaments form an elaborate array throughout the cytoplasmic space and often terminate in membrane-bound desmosomes.[1] Desmosomes form intercellular junctional complexes and essentially link the cytokeratin filament system of one cell to that of its neighbor.[5] This system of linked cytokeratin filaments presumably strengthens the epithelial cell sheet against mechanical insult and deformation.[5] In humans, there are 20 known subtypes of cytokeratins, based on their polypeptide composition.[8,9]

The distribution of intermediate filaments within the inner ear has only recently been investigated by

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different laboratories. Most studies have focused on the cochlea and organ of Corti, and few studies have investigated the vestibular organs. The absence of intermediate filaments within hair cells of the organ of Corti and their presence within nonsensory epithelial cells have been documented.[10-12]

The auditory and vestibular organs develop metachronously from the otic placode. The auditory system is phylogenetically younger than the vestibular system and may differ in its ultrastructural composition. It is the purpose of this study to examine the distribution of a cytokeratin class of intermediate filaments in the vestibular epithelium of the guinea pig.

METHODS

Ten pigmented guinea pigs weighing 250 to 400 g were used. Animals were anesthetized with an intraperitoneal injection of 10 mg/kg Beuthanasia-D with subsequent cardiac perfusion of a 4% solution of paraformaldehyde in 0.1 mol/L phosphate buffered saline (PBS), pH 7.4. The temporal bones were then harvested, the bulla opened, and the stapes removed. Inner ear perfusion with fixative was then performed. After fixation the osseous otic capsule was removed piecemeal by dissection. The vestibular sensory epithelium was harvested as a whole tissue surface preparation with the aid of a dissecting microscope. Cochleae from these animals were processed simultaneously as control tissue, since the distribution of cytokeratins in the cochlea has been previously described.[10-12] The crista ampullaris of each canal (lateral, superior, and posterior), utricle, and saccule was studied.

Six animals were processed for fluorescence immunohistochemistry. The tissue was permeabilized with 0.3% Triton X-100 for 10 minutes. This permeabilization regimen is routinely used in our laboratory to prepare inner ear epithelia for immunocytochemistry. It consistently allows intracellular localization of different cytoplasmic proteins. Specimens were then incubated with 4% normal goat serum for 30 minutes to block nonspecific antibody binding. After rinsing with PBS, the tissue was incubated for 1.5 hours with monoclonal pan-anticytokeratin antibody (Boehringer Mannheim), which binds to an epitope common to all cytokeratins. Specimens were then washed and incubated with rhodamine-conjugated goat anti mouse IgG for 30 minutes. Fluorescein isothiocyanate–conjugated phalloidin was used at a 1:75 dilution in combination with the secondary antibody to double-label for actin. The tissue was then washed again and mounted on a welled microscope slide in glycerol-PBS solution. Specimens were examined, as whole mounts, with a Leitz Orthoplan microscope equipped for epifluorescence and a Biorad laser confocal microscope. Photomicrographs of specimens were taken with Kodak T-max films.

Three animals each were processed for sectioning for light microscopy (LM) and electron (EM) microscopy. Specimens were obtained as whole tissue preparations as described above for fluorescence immunohistochemistry; however, the immunoperoxidase technique was used to label cytokeratins. Specimens were permeabilized with 0.3% Triton X-100, rinsed, and incubated with 5% normal horse serum to block nonspecific antibody binding. Specimens were then treated with the anticytokeratin antibody, rinsed with PBS, and incubated with the second antibody for 1.5 hours. The second antibody used was biotinylated and affinity-purified. The biotinylated secondary antibody was then detected by means of a preformed macromolecular complex between avidin and the biotinylated enzyme, employing the Vectastain ABC Kit from Vector Laboratories (Burlingame, Calif). In control specimens, labeling was as described above, except for omitting the anticytokeratin antibody.

The specimens were postfixed in 1% osmium tetroxide, dehydrated in alcohol, embedded in Epon 812, and sectioned with a glass knife or diamond knife (for LM and EM, respectively) on an LKB ultramicrotome. Semithin sections for light microscopy were stained with toluidine blue, examined, and photographed with a Leitz Dialux microscope. Thin sections of 90 nm were stained with uranyl acetate and lead citrate, and examined with a JEOL JEM 1200 EX electron microscope.

RESULTS

Examination of the vestibular sensory epithelium reveals a similar pattern of cytokeratin and actin staining compared to the auditory system (Figs 1 and 2). In both the organ of Corti and vestibular neuroepithelium, actin-specific staining in hair cells is seen in the stereocilia and cuticular plate and in association with the circumferential ring of adherent junctions at their apical surface. This represents the reticular membrane of the cells. An actin-free zone is seen within the cuticular plate, representing the position of the basal body in both vestibular and auditory hair cells. Actin is also seen forming a band around the apical surface of supporting cells of the auditory and vestibular sensory epithelium. This represents actin in the reticular lamina of supporting cells (Figs 1A, 2A, and 3). Cytokeratin immunolabeling was restricted to the supporting cells of the vestibular and auditory epithelium (Figs 1B and 2B). There is a

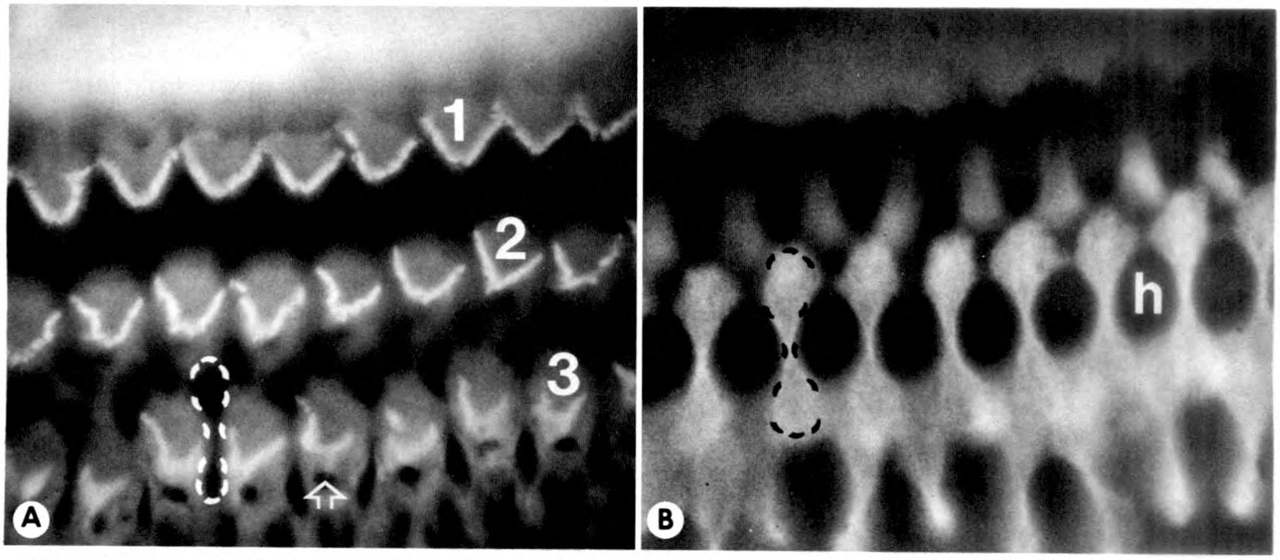

Fig 1. Whole mount of organ of Corti, double-labeled with A) fluorescent phalloidin and B) cytokeratin-specific antibodies (original ×1,350). A) Actin-specific staining is seen in stereocilia and cuticular plate of hair cells (1, 2, 3 are hair cells in first, second, and third rows of outer hair cells, respectively), and in intercellular junctions at reticular lamina. Actin-free zone is seen within cuticular plate, representing position of basal body (open arrow). Apical surface of supporting cells appears as dumbbell shape (broken line) interspersed between hair cells. B) Cytokeratin immunostaining is confined to apical surface of supporting cells, which forms dumbbell shape (broken line). Supporting cells are interposed between hair cells (h). Hair cells lack cytokeratin staining.

complete absence of cytokeratin staining of auditory and vestibular hair cells as viewed from above in whole tissue mounts. This was found to be true in all five vestibular sense organs.

Specimens stained for cytokeratin were sectioned and examined with light and electron microscopy. With light microscopy, cytokeratin staining was found to be present within supporting cells of the vestibular epithelium (Fig 4A). Cytokeratin staining was seen throughout the supporting cells and had its greatest intensity at the apical surface. There was a complete lack of cytokeratin staining in the vestibular hair cells at the light microscopic level.

These same specimens were also examined by electron microscopy. Peroxidase staining could be identified on electron micrographs as an electron-dense reaction product within cells (Fig 5A). Cytokeratin staining was only present in the cytoplasmic domain of supporting cells, and not within hair cells. Cytokeratin staining had its greatest intensity near

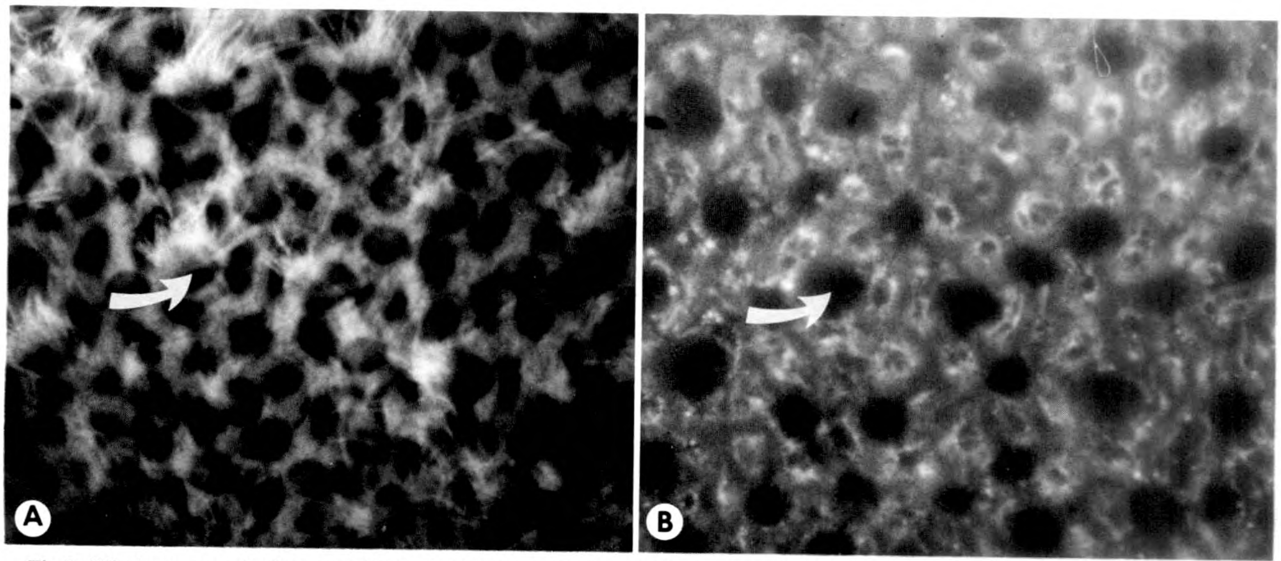

Fig 2. Whole mount of crista ampullaris, double-labeled with A) fluorescent phalloidin and B) cytokeratin-specific antibodies (original ×1,350). Arrows — same vestibular hair cell. A) Actin-specific staining is seen in stereocilia and cuticular plate of hair cells, and in intercellular contacts. B) Same field as in A, viewed for cytokeratin immunostaining. Cytokeratin labeling is confined to supporting cells, whereas hair cells, devoid of label, appear as dark circles (arrow).

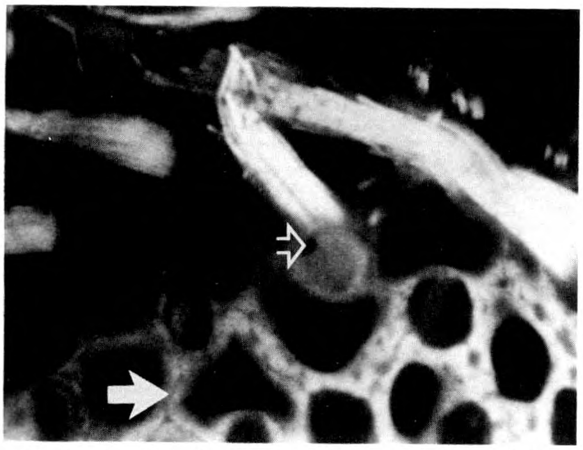

Fig 3. Laser scanning confocal micrograph of vestibular hair cells labeled with fluorescent phalloidin. Actin staining is present within stereocilia and cuticular plate. Actin-free zone is seen within cuticular plate, representing region around basal body (open arrow). Slender line, lacking actin label, represents intercellular space between adjacent cells (filled arrow).

the reticular membrane of supporting cells.

Desmosomes were identified in type 2 hair cells of the vestibular epithelium (Fig 5B). Desmosomes, which form a linkage between cells, are associated with cytokeratin, which forms a "brush" on each side of the junction. Desmosomes are thickened regions of the plasma membrane and act to anchor tightly one cell to its neighbor. Hence, it appears that cytokeratins are absent within the cytoplasmic domain of hair cells but are present in association with intercellular junctions.

The nonsensory epithelium of the vestibular labyrinth was also investigated for the distribution of cytokeratins. The transitional cells of the vestibular epithelium are located at the base of the crista ampullaris and are columnar in shape and convex at the basal epithelium. These cells contain large nuclei and have multiple fingerlike projections between neighboring cells[13] (Fig 4B). The cytokeratin staining of these cells was unique. The cytokeratin staining of transitional cells had its greatest intensity in both the apical and basal portions of the cell, as observed by light and electron microscopy (Figs 4B and 5C).

Dark cells of the vestibular epithelium, felt by some authors to be the secretory cells that produce endolymph,[14,15] also contained cytokeratin. These cells are flat toward their luminal surface and have multiple cytoplasmic projections on their basal surface. The cytoplasm is densely populated with mitochondria and endoplasmic reticulum. The nuclei are irregularly shaped, with multiple invaginations. Cytokeratin expression was greatest in intensity at the apical surface of dark cells (Fig 5D).

DISCUSSION

Cytokeratins in the mammalian auditory sensory epithelium are present within nonsensory cells, but are absent from hair cells of the organ of Corti.[10-12] Our current study confirms this differential distribution of cytokeratins within the organ of Corti. Several studies have examined the developing otocyst and documented the presence of cytokeratins within the epithelium of the future sensory organ. There is a loss of cytokeratin from the hair cell after differentiation of the otocyst. This has been documented in humans, guinea pigs, and mice.[10,16-18]

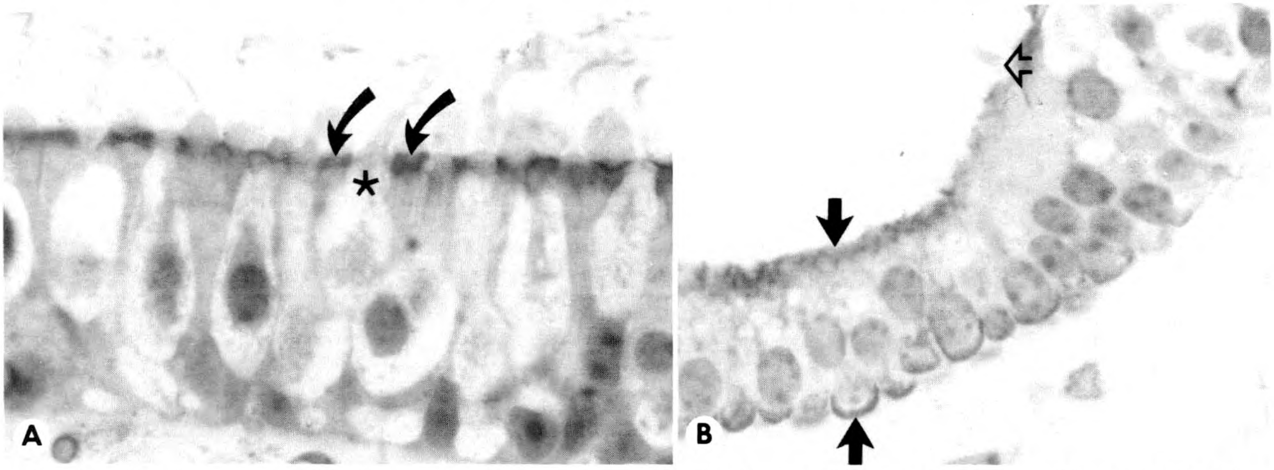

Fig 4. Light micrographs. A) Plastic-embedded crista ampullaris, sectioned longitudinally and labeled for cytokeratins (original ×1,350). Type 1 hair cells are surrounded by nerve chalices containing numerous mitochondria. Supporting cells contact basement membrane and extend long and slender neck region to reach apical (luminal) surface. Cytokeratin immunostaining is found only within supporting cells, and their apical surface is densely labeled (arrows). Cytoplasm of hair cells is devoid of cytokeratin staining (asterisk). B) Transitional cells at base of crista ampullaris, labeled for cytokeratins (original ×560). Hair cell is seen at upper right hand portion of micrograph (open arrow). Transitional cells are immunostained with cytokeratins. Staining is greatest in intensity at both apical and basal portions of transitional cells (filled arrows).

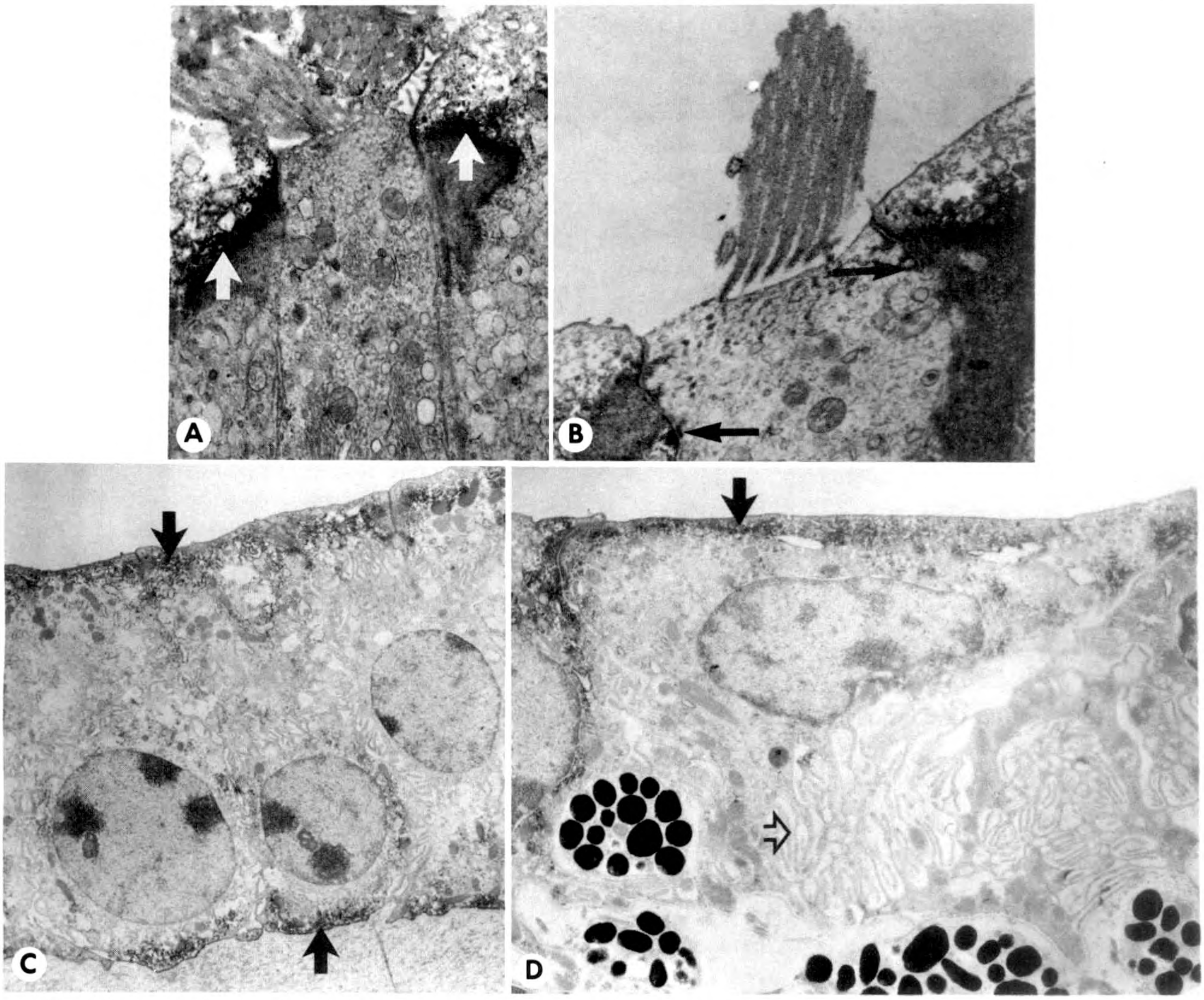

Fig 5. Electron micrographs. A) Plastic-embedded crista ampullaris sectioned longitudinally and labeled for cytokeratins (original ×12,000). Type 2 hair cell, lacking nerve chalice, is seen with stereocilia extending from its apical surface. Hair cell is surrounded on either side by supporting cells. Peroxidase staining of cytokeratin is represented by electron-dense reaction product within supporting cells (arrows). Cytokeratin staining was not present in cytoplasmic domain of hair cells. B) Type 2 vestibular hair cell with two adjacent supporting cells (original ×18,000). Cytokeratin label is seen within cytoplasmic domain of supporting cells. Desmosomes are identified within type 2 hair cells (arrows). C) Transitional epithelium stained with cytokeratin-specific antibodies (original ×6,000). Peroxidase staining of cytokeratins has its greatest intensity at both apical and basal borders (arrows) of transitional cells. D) Dark cell epithelium stained for cytokeratins (original ×9,500). Dark cell is seen with typical basal infolding (open arrow). Pigment cells are also seen in this area. Cytokeratin expression is greatest in intensity at apical surface of dark cells (filled arrow).

Cytokeratins were identified previously in the vestibular sensory epithelium of humans, but the differential distribution between hair cells and supporting cells was not reported.[19] Wikström et al[18] reported that both hair cells and supporting cells express cytokeratin in the vestibular epithelium of the mouse. Thornell et al[20] and Anniko et al[16,21] report that hair cells of the vestibular sensory epithelium of human fetuses lack all intermediate filament expression, and only supporting cells were noted to express cytokeratin and vimentin. Anniko and Arnold[17] noted the presence of cytokeratin in epithelial cells of the human otocyst and the absence of cytokeratin in hair cells of adult vestibular tissues. In contrast, Anniko et al[21,22] were able to identify cytokeratin expression in hair cells of the vestibular sensory epithelium of the human, the CBA mouse, and the jerker mouse mutant. They were able to identify cytokeratins 8 and 18 in hair cells of the vestibular epithelium. Not all antibodies against the same cytokeratin subtype identified cytokeratin in hair cells of the vestibular epithelium. Anniko et al[21] ascribed the differing results to the possibility that antigenic sites for the monoclonal antibody were blocked and not accessible to the antibody.

All previous reports of cytokeratin expression in the vestibular epithelium used light microscopy to study sectioned tissues. The present study attempts to clarify the issue of cytokeratin expression in the vestibular sensory epithelium, using immunofluorescence microscopy and surface preparations, viewing the entire epithelium as a whole mount. We were able to clearly differentiate between hair cells and supporting cells on the basis of their distinct morphology and unique staining pattern for actin. We used monoclonal pan-anticytokeratin, which identifies an epitope of cytokeratin common to all subtypes. With this method we were able to determine that hair cells of the vestibular sensory epithelium lacked cytokeratin expression in their cytoplasmic domain as viewed from above on surface preparations. We also investigated cytokeratin expression in sections of the vestibular epithelium by light and electron microscopy. By light microscopy there was a complete absence of cytokeratins within hair cells of the vestibular epithelium. Electron microscopy revealed the presence of desmosomes in type 2 hair cells of the vestibular epithelium. The presence of desmosomes attests to the presence of cytokeratins in association with intercellular junctions of the type 2 hair cell. This apparent lack of cytoplasmic cytokeratins and the presence of desmosomes may explain the controversial reports in the literature on the expression of cytokeratin in the vestibular epithelium. The minute amounts of cytokeratin anchored to desmosomes may render them unidentifiable by current immunofluorescence techniques.

Gap junctions are believed to act as an electronic coupler allowing ions and electrical currents to pass from one cell to the other.[23] Gulley and Reese[23] studied the intercellular junctions in the chinchilla organ of Corti with electron microscopy and freeze-fracture techniques. Gap junctions were not present within hair cells of the organ of Corti. Gap junctions were present at the opposition sites between two supporting cells. Thus, supporting cells of the organ of Corti are coupled to each other, but not to hair cells. Thornell et al[20] also state that the expression of cytokeratin is dependent on extensive intercellular interactions. These authors believe that the hair cell is completely uncoupled from surrounding supporting cells and nonsensory cells, in both the cochlea[23] and vestibule.[20] The presence of desmosomes within hair cells of the vestibular epithelium of the guinea pig provides direct evidence of linkage between vestibular hair cells and supporting cells.

The lack of cytokeratins within the cytoplasmic domain of vestibular hair cells is intriguing. Anniko and Arnold[17] theorized that the abundance of cytokeratin in supporting cells surrounding the vestibular hair cell would form a protective shell around the hair cell. The hair cell would then be protected from mechanical influences at its lateral and basal surfaces. Only the apical surface would be exposed to mechanical vectors. Bauwens et al[19] proposed that cytokeratin expression in supporting cells of the vestibular epithelium renders the epithelium rigid in comparison to the overlying otoconial layer of cupula. The linking of type 2 hair cells to supporting cells most likely adds rigidity and stiffness to the vestibular epithelium, so that only the stereocilia would be influenced by mechanical stimulation.

We also documented the presence of abundant cytokeratin in the apical and basal portions of transitional cells near the crista ampullaris. Cytokeratins and intermediate filaments in general are the most stable component of the cytoskeleton. We speculate that these strategically placed cytokeratins within transitional cells may be necessary to maintain the rigidity of the crista ampullaris and to prevent mechanoelectrical transduction from forces other than those affecting the stereocilia. Future studies will be necessary to determine the role of cytokeratins at the apical surface of the dark cells.

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IMAGING CASE STUDY OF THE MONTH

MALIGNANT HEMANGIOENDOTHELIOMA OF THE TEMPORAL BONE MASQUERADING AS GLOMUS TYMPANICUM

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BRIAN C. BOWEN, MD, PHD
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Malignant hemangioendothelioma is a rare tumor of vascular origin involving bone or soft tissue. It is characterized by groups of irregular vascular elements lined by anaplastic, immature-appearing endothelial cells.[1] This lesion has a multitude of synonyms, including angiosarcoma,[2] hemangiosarcoma,[3] angioblastoma,[2] and intravascular endothelioma,[3] which have led to confusion in the classification and case reporting. Although the bones of the limbs are most commonly involved,[3] numerous cases of craniofacial and head and neck involvement have been reported, including sites in the mandible,[2-5] maxilla,[1,6-10] frontal bone,[3] and temporal bone.[11,12]

Lesions of the temporal bone are extremely rare, with only two prior reports of mastoid[11] or petrous[12] involvement appearing in the English-language literature. The purpose of this paper is to present a case of malignant hemangioendothelioma involving the middle ear space, originally believed to be glomus tympanicum, and a review of the current literature.

CASE REPORT

A 62-year-old man presented complaining of asymmetric nonpulsatile tinnitus (left greater than right) starting 5 weeks earlier, associated with left ear fullness and a sense of imbalance, not described as true vertigo. The tinnitus had increased over the previous few weeks, requiring self-medication with benzodiazepine for sleep. There was no history of prior otologic infection, trauma, or ototoxicity. His medical history was significant for coronary artery disease requiring previous angioplasty, hypertension controlled by medication, and a past cerebrovascular accident. Medications taken included calcium channel blockers, antiarrhythmics, and diuretics.

On physical examination, the ear canals and tympanic membranes were normal bilaterally, with normal mobility, and no masses were noted. The remaining otolaryngic and neurologic examination revealed no disease.

Audiometric examination revealed a slightly asymmetric, bilateral, high-frequency sensorineural hearing loss at 2 to 4 kHz (left worse than right), a speech reception threshold of 10 dB hearing level (HL) on the left and 5 dB HL on the right, and speech discrimination 92% at 50 dB HL on the left and 100% at 45 dB HL on the right. Immittance audiometry was not performed.

Magnetic resonance imaging of the head with and without intravenous gadolinium had been performed prior to referral. On the precontrast, T2-weighted images (see Figure, A), an elongated, 1-cm area of mixed signal intensity, isointense anteriorly and hyperintense (compared to brain parenchyma) posteriorly, was identified in the region of the left cochlear promontory and the basal turn of the cochlea. On the T1-weighted image (see Figure, B), the anterior and posterior components were hypointense and isointense, respectively. Following intravenous gadolinium administration, the abnormal area, particularly the posterior component, demonstrated marked enhancement on T1-weighted images (see Figure, C,D). A subsequent arteriogram was normal.

Left middle ear exploration 1 month later revealed a pulsatile, reddish mass surrounding Jacobsen's nerve, extending to the protympanum, round window, sinus tympani, and oval window, with erosion of the bone overlying the auditory tube. The lesion was thought to be completely excised, but margins were not controlled by frozen section biopsy, to avoid violating the cochlea and because the appearance was thought to be consistent with glomus tympanicum. Microscopic examination revealed a mesenchymal spindle cell tumor characterized by small endothelial-lined spaces consistent with the diagnosis of hemangioma, which was the final pathologic diagnosis.

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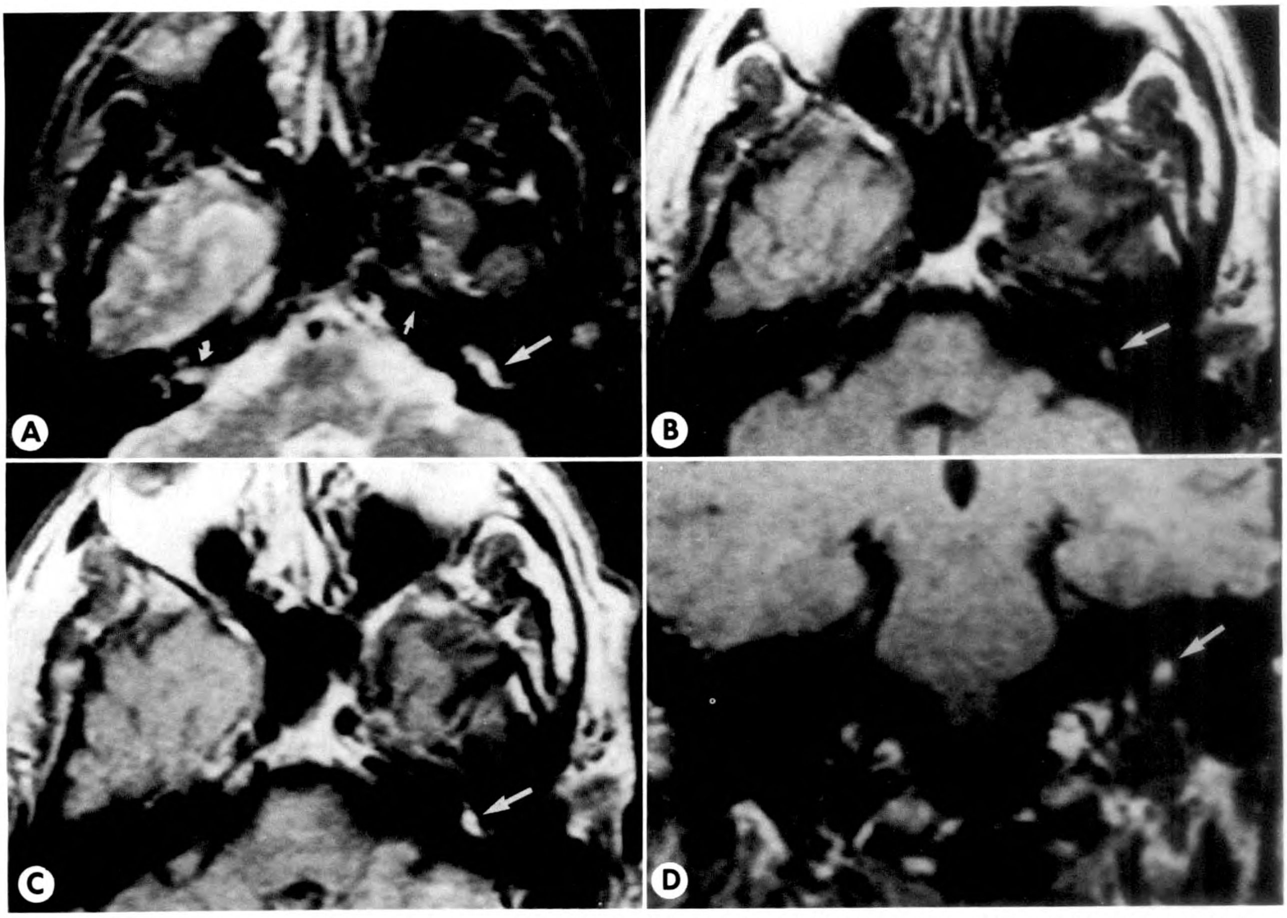

Magnetic resonance images of temporal bones before and after intravenous gadolinium. A) On axial T2-weighted image (and subsequent images), patient's head is slightly tilted, showing level of horizontal segment of internal carotid artery ("flow void," short, straight arrow) on left and level of internal auditory canal (curved arrow) on right. Abnormal signal in region of left basal turn of cochlea and cochlear promontory has hyperintense component posteriorly (long, straight arrow). B) On precontrast axial T1-weighted image, component of hyperintense signal in A is seen to be isointense (arrow). On postcontrast C) axial and D) coronal T1-weighted images, component of abnormal signal identified in A and B demonstrates marked enhancement (arrows).

Five months postoperatively, the patient developed severe vertigo associated with nausea and vomiting, which was treated with benzodiazepine and Cawthorne exercises with no resolution. An audiogram revealed anacusis on the left, and an electronystagmogram revealed an absence of caloric responses.

A computed tomography scan of the temporal bones performed at that time revealed a soft tissue density in the left posterior mesotympanum, with fistulization of the left horizontal semicircular canal, extension into the vestibule through the oval window, and involvement of the tympanic segment of the facial nerve.

Subsequently, the patient underwent a left subtotal petrosectomy, translabyrinthine labyrinthectomy, and ear obliteration, at which time a reddish, vascular mass was identified, infiltrating the mastoid antrum and eroding through the posterior canal wall to involve the fallopian canal and the horizontal semicircular canal, resulting in fistulization. No tumor was noted in the vestibule. Because of the prior benign pathologic diagnosis and the patient's wishes, in spite of a preoperative suspicion of malignancy, gross tumor was removed from the facial nerve and a portion of the sheath was resected, but the nerve was not sacrificed to ensure total tumor removal.

Final pathology revealed a vasoformative spindle cell lesion characterized by slitlike vascular spaces lined by plump endothelial cells with a narrow range of pleomorphism and bony invasion consistent with a low-grade angiosarcoma (malignant hemangioendothelioma). Reexamination of the original specimen revealed similar pathologic findings.

Consultation with the radiation oncology department was obtained postoperatively, and the patient received 5,580 cGy over 8 weeks. Magnetic resonance imaging scans performed approximately 3 months and 12 months after radiotherapy revealed no evidence of tumor. The patient is currently stable but still complaining of moderate vestibular symptoms, for which he is receiving vestibular exercises.

DISCUSSION

Although vascular neoplasms involving the temporal bone have been recognized for many years, the inadequate classification scheme and conflicting terminology have led to an ambiguous histologic definition resulting in scattered case reports in the literature. A review of the cases reported in the late 19th and early 20th centuries reveals that many of the lesions reported as hemangioendothelioma would not be classified as such today, bringing into question the actual incidence of this lesion during this period. The modern classification scheme had its beginnings in 1908, when Mallory[13] introduced the term hemangioendothelioma to describe these lesions, and was further advanced with reports of true vascular bone tumors. Eventually, Thomas[14] studied both benign and malignant lesions of bone and proposed that malignant lesions with the endothelial cell as the proliferating neoplastic unit be classified as angioendotheliomas. Stout[15] further proposed that hemangioendotheliomas could be either benign or malignant, with atypical endothelial cell proliferation being used to define malignancies.

Currently, vascular lesions have many classification schemes. Angiosarcoma can be used as the term to describe any malignant vascular tumor, and malignant hemangioendothelioma is considered a subclassification. These lesions can be divided into low-, intermediate-, and high-grade malignant variants. Low- to intermediate-grade lesions are defined as hemangioendothelioma (epithelioid, spindle cell type, Dabska tumor), and high-grade lesions are considered conventional angiosarcomas. Others classify all malignant lesions as hemangioendothelial sarcomas with various degrees of malignancy.[3]

Pathologic features characterizing malignant hemangioendothelioma have a wide variation and include a proliferation of capillaries with pleomorphic, hyperchromatic cells in the endothelial lining.[3,5] Irregular, interanastomosing vascular channels are lined by plump, pleomorphic cells that can extend into the stroma or surrounding tissue as the lesion enlarges.[1,3] These tumors stain positive for factor VIII–related antigen.[5] Frequently, as seen in this case, the lesion may initially be mistaken for hemangioma because of the variation in microscopic appearance.[6]

Although classified as malignancies, hemangioendotheliomas may initially behave as benign pathologic entities with insidious growth patterns and cause symptoms by infiltration and space-occupying dynamics. Angiosarcomas of osseous origin are thought to be less aggressive than those arising in the soft tissues. Spread of disease is mainly by local extension, although regional and distant metastasis has been reported.[1]

Malignant hemangioendothelioma is considered a rare vascular tumor, representing fewer than 1% of primary malignancies of bone. The number of cases reported varies according to the classification scheme used, but the overall incidence appears to be less than 1%, with equal gender distribution. The cause is unknown; however, there have been case reports associated with trauma, irradiation, and angiectasia. The overall prognosis is considered poor. Both unifocal and multifocal (monostotic and polyostotic) disease has been reported, with the multifocal more common in the limbs, and unifocal more common in the craniofacial skeleton. The most common site of involvement is the limb skeleton (femur, humerus, tibia, and pelvis).[3]

Treatment protocols usually consist of combined therapy, with wide surgical excision and control of margins and postoperative radiotherapy. These tumors are considered radiosensitive, but do not usually respond to irradiation alone.[1] Radiotherapy may be considered preoperatively to reduce the size of the lesion, or may be given alone for palliation when the patient is medically unstable or unresectable.[4] Chemotherapy has not proved effective, either in combination with other modalities or as the primary treatment. Neck dissection is indicated if regional metastasis has occurred.[1]

Reports of lesions involving both the soft tissues and skeleton of the head and neck have appeared in the literature. Panje et al[4] reported 11 cases involving the head and neck region, and noted that the soft tissue regions most commonly involved were the scalp and facial skin, while osseous lesions most commonly involved the skull and the mandible. Ellis and Kratochil[5] reported 12 cases involving soft tissues only, and reported the submandibular region as the most commonly involved. Other regions involved included the submental and posterior triangles of the neck, along with the ethmoid sinus, maxilla, and mandible. Most cases presented as a darkened, painless, soft skin lesion with indistinct margins. Treatment consisted of complete surgical excision with control of frozen section margins and, occasionally, postoperative radiotherapy. Survival statistics were not available because of the small number of patients.[4,5]

Lesions involving the nasal cavity, paranasal sinuses, and nasopharynx are also considered very rare, with scattered reports available,[7] most describing involvement of the maxilla and maxillary antrum.[1,6-10] Presenting symptoms include nasal obstruction, headache, facial pain and swelling, epistaxis, and displacement of the orbit. Surgical excision, with or without postoperative radiotherapy, is the treatment of choice; however, radiotherapy can be used alone for palliation.

The case presented in this paper represents the third reported case of temporal bone involvement in the English-language literature. As in previous cases, the initial presentation included tinnitus and imbalance. Other presenting symptoms include otalgia, aural discharge, fullness, hearing loss, and mastoid tenderness.[11,12] The treatment in this case, consisting of total gross resection but sparing of the adjacent facial nerve and postoperative radiotherapy, was also used in one of the two previous cases, with excellent early results.[11] In the other case, the patient received radiotherapy alone because of the extent of the disease, and died 9 months after presentation.[12]

This was the first malignant hemangioendothelioma reported in the temporal bone to have been studied with magnetic resonance imaging. The lesion was noted to be of mixed signal intensity on precontrast T1- and T2-weighted images (see Figure, A,B), reflecting either heterogeneity of the tumor and/or partial volume averaging. With the administration of intravenous gadolinium, the T1-weighted images showed marked enhancement (see Figure, C,D). The differential diagnosis for the above findings includes paraganglioma (glomus tympanicum), hemangioma (hemangioendothelioma), schwannoma, granulation tissue, and vascular pseudotumor (aneurysm or vascular malformation or fistula). As a result of the above imaging results, our original impression of the lesion was consistent with glomus tympanicum, and it was not until the final pathology that the diagnosis of malignant hemangioendothelioma was made.

In conclusion, malignant hemangioendothelioma is a malignant tumor originating in vascular elements involving both soft tissue and bone. Occurrence in the temporal bone, along with other regions of the head and neck, is extremely rare. Radiographic diagnosis can be misleading because of the rarity of the tumors, and usually a pathologic specimen is needed for definitive diagnosis. Treatment protocols usually consist of combined surgical excision with postoperative radiotherapy, and the overall prognosis is poor.

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IMAGING CASE STUDY OF THE MONTH

OSTEOSARCOMA METASTATIC TO THE LARYNX

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INTRODUCTION

Secondary laryngeal tumors are uncommon, accounting for approximately 0.02% of all laryngeal malignancies.[1,2] In 1988, Ferlito et al[1] reviewed the world literature, which revealed 113 cases, and his series added another 7, for a total of 120 cases known at that time. We have identified 5 additional cases that have been reported.[3-7] The most common primary tumors metastasizing to the larynx are melanomas of the skin (27% to 37%), renal cell carcinomas (13% to 17%), carcinomas of the breast (9% to 11%), lung carcinomas (8% to 11%), and adenocarcinomas of the prostate (6% to 7%).[1,2,4] The following is the first case report known to us of an osteosarcoma metastatic to the larynx.

CASE REPORT

A 51-year-old man presented with a right neck mass that was found to be osteogenic sarcoma metastatic to the larynx. Ten years earlier, he had undergone an above-the-knee amputation for an osteogenic sarcoma of the right proximal tibia (Fig 1A,B). Two years later, he was found to have a solitary right pulmonary metastasis, which was resected without difficulty. He did well for an additional 5 years, then developed a metastasis to the distal left femur. He received chemotherapy and a wide block resection of the distal femur with endoprosthetic replacement. Two years later, he was found to have a metastasis to the right side of the neck, confirmed by fine-needle biopsy (Fig 1C). The neck lesion was found by magnetic resonance imaging (MRI) scan to involve the thyroid cartilage and adjacent soft tissue, and on indirect laryngoscopy, the lesion was not visualized internally. The mass was resected with part of the thyroid cartilage and adjacent soft tissues, sparing both recurrent and superior laryngeal nerves. The patient received postoperative radiotherapy to the right side of the neck of 160 cGy twice daily to a prescribed dose of 6,880 cGy. Simultaneous to the neck mass, a subcutaneous metastasis to the left flank was found and widely excised. On last follow-up, the patient is 1 year from completion of his irradiation treatments and is doing well, without evidence of disease.

MAGNETIC RESONANCE IMAGING

The MRI scans revealed a lesion on the right side of the larynx, involving the thyroid cartilage and the adjacent soft tissues of the neck. The mass appeared to originate from the marrow space on the left side of the thyroid cartilage, which was destroyed by the lesion (Fig 2A). There was preservation of the right side of the thyroid cartilage and of the entire cricoid cartilage. On coronal sections, the vocal cords appeared displaced medially without invasion, and comparing T1- and T2-weighted images led us to conclude that the lesion was submucosal (Fig 2B,C).

DISCUSSION

Metastasis to the larynx is an uncommon event,[1,2] and metastases occurring in the thyroid cartilage are rarer still, contributing 2.6% of secondary laryngeal tumors.[1] Metastases to the thyroid cartilage are possible only after endochrondral ossification and development of a marrow space, which occurs by adulthood.[8] Their most likely route of spread is via tumor emboli to the thyroid cartilage hematogenously, either retrograde through the valveless vertebral veins, as described by Batson,[9] or from the vena cava through the pulmonary circulation and finally the laryngeal artery.[10]

Metastasis to the larynx is almost always associated with long-standing, widely disseminated disease, and carries a poor prognosis.[6,11] General treatment recommendations for these lesions have em-

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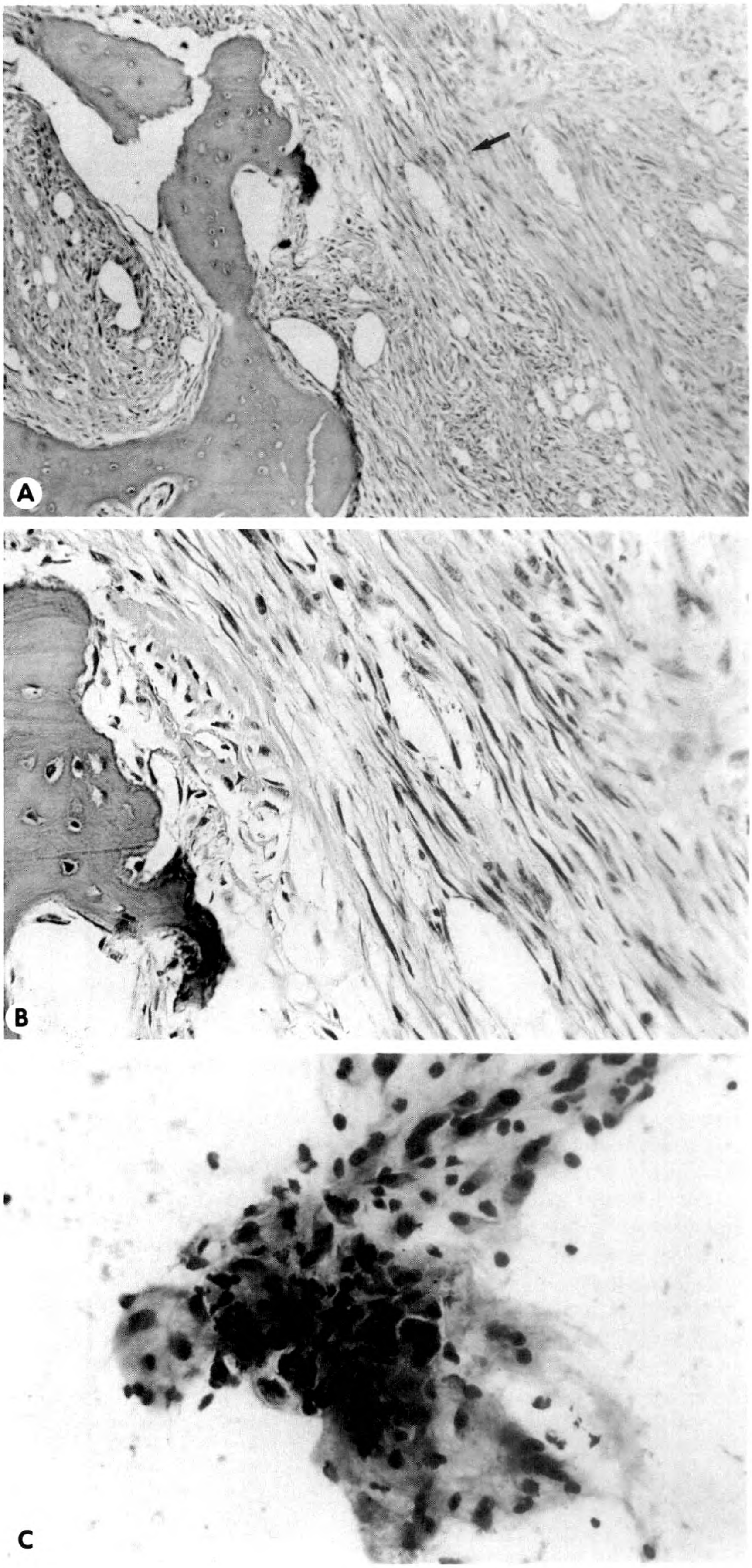

Fig 1. Histopathologic findings. A) Highly cellular spindle cells (arrow) infiltrating marrow space (H & E, original ×150). B) Individual spindle cells have elongated, relatively uniform nuclei embedded in collagenous background, supporting diagnosis of well-differentiated osteosarcoma dominated by fibrosarcomatous elements (H & E, original ×400). C) Fine-needle aspiration demonstrated group of round to oval cells embedded in fibrous stroma (H & E, original ×400). Nuclei are irregular and hyperchromatic, suggestive of metastatic tumor.

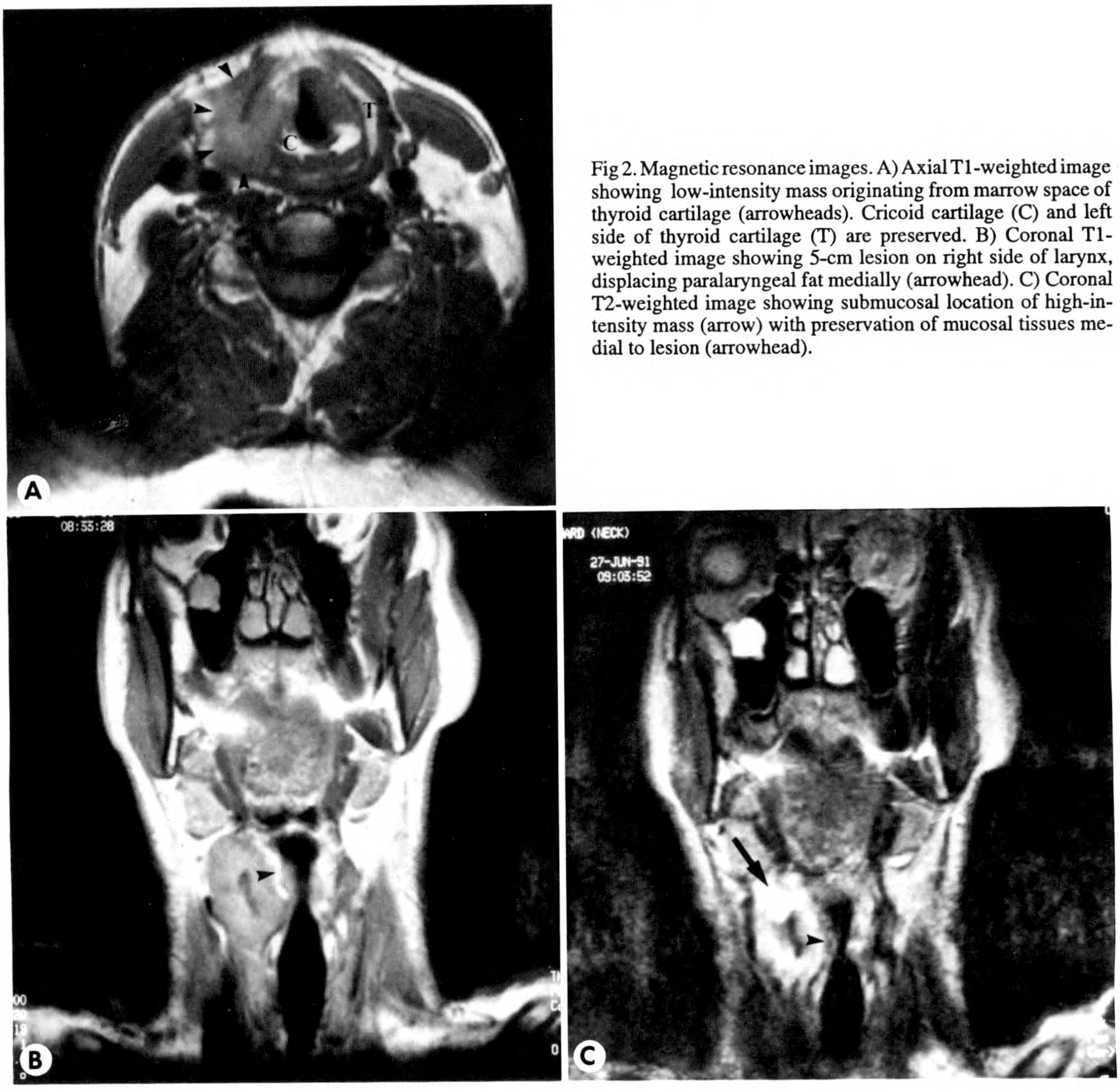

Fig 2. Magnetic resonance images. A) Axial T1-weighted image showing low-intensity mass originating from marrow space of thyroid cartilage (arrowheads). Cricoid cartilage (C) and left side of thyroid cartilage (T) are preserved. B) Coronal T1-weighted image showing 5-cm lesion on right side of larynx, displacing paralaryngeal fat medially (arrowhead). C) Coronal T2-weighted image showing submucosal location of high-intensity mass (arrow) with preservation of mucosal tissues medial to lesion (arrowhead).

phasized a palliative approach,[1,6,11,12] but there are reports in the literature of long-term survivors.[13-15] In the case of metastatic osteosarcoma, there are reports in the literature promoting metastatectomy in selected patients, especially those with isolated pulmonary metastases. In those patients treated surgically without evidence of disease, there is a 20% to 30% long-term survival.[16,17] The principle of metastatectomy could be extended to less common sites of osteosarcoma metastases, including the larynx, in light of changing patterns of disseminated disease with the advent of aggressive chemotherapy.[18]

This case report illustrates the typical long-standing history of patients found to have metastases to the larynx. To our knowledge, it also represents the first case in the literature of an osteosarcoma metastatic to the larynx.

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PATHOLOGY CONSULTATION

ANEURYSMAL BONE CYSTS OF THE JAWS

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HOUSTON, TEXAS

Aneurysmal bone cysts of the jaws are histologically the same as those found in the more usual appendicular sites for this lesion. They may be primary or secondary (associated with another definable osseous lesion) and have a 2:1 or 3:1 predilection for the mandible over the maxilla. Curettage is associated with relatively high recurrence rates, while surgical excision, with negative margins, of primary lesions markedly reduces this incidence.

The aneurysmal bone cyst (ABC) is a nonneoplastic, presumably reactive lesion of bone and exists in two clinicopathologic forms: either as a primary lesion or as a secondary lesion arising in other osseous conditions (see Table).[1,2] Approximately 30% of ABCs are considered to be secondary, and these are most often found in long bones.[1] More than 80% of primary ABCs are in long bones, flat bones, or the spine, where they have an incidence estimated to be half that of giant cell tumors.[2] In a Mayo Clinic series only five (2.1%) of 238 primary ABCs involved the jaw bones.[2] Six others were in the extragnathic skull. These data reflect the experience of others.[3-6]

Regardless of the bone, primary ABC is mainly a lesion of children and young adults. In the jaws, there is a predilection for the mandible, particularly its body (20 cases or 29.4% in the maxilla and 48 cases or 70.6% in the mandible).[5,6] There is a modest female predominance. A localized, expansile mass, producing facial deformity and malocclusion and often associated with pain, is the principal clinical finding. There are no bruits. Radiographic findings are not diagnostic. There is often a circumscribed, lytic, soap-bubble or honeycomblike lesion with a sclerotic peripheral border due to reactive new bone.

The cause and pathogenesis of ABC are unknown. None of the prevailing theories — posttherapeutic reparative, altered hemodynamics (eg, arteriovenous fistula), or secondary to compression by another osseous lesion — satisfy all cases.

Bertoni et al[7] have appropriately indicated that primary ABC presents with a spectrum of gross and histomorphological features. The "classic" form is one with cavernous vascular spaces, separated by variably sized fibrous septae, accompanied by stromal giant cells and strands of bone (see Figure). This is also reflected in the gross appearance in that there is a spongy, blood-filled lesion. At the opposite pole of the spectrum is the solid variant (5% of ABCs), in which the vascularity is inconspicuous or in the form of small spaces lined by a cellular fibrous tissue and giant cells. A rather florid fibroblastic or fibrohistiocytic proliferation with scattered osteoclastlike giant cells is the prominent feature. Osteoblastic differentiation with osteoid and foci of a calcifying fibromyxoid tissue complete the picture. This latter finding, although not always seen, is regarded by some as a reliable diagnostic feature. Again, the gross appearance reflects the microscopic, with the lesion being solid, fleshy, and fibrous, with scattered hemorrhagic zones. Bridging the cavernous and solid types is a transitional or mixed ABC showing features of both.

A secondary ABC has been likened to a "blowout" in a preexisting lesion of bone, the most often recorded being fibrous dysplasia, giant cell tumor, chondroblastoma and osteoblastoma, and osteosarcoma.[1,2,4] The frequency of the association is a variable of the amount of tissue examined and perhaps, too often, a too-liberal definition of ABC. To date, secondary ABCs of the jaws have been associated only with nonmalignant tumors.

ANEURYSMAL BONE CYSTS OF JAWS DIVIDED INTO PRIMARY AND SECONDARY FORMS

- Primary (histologic variants)
  - Vascular
  - Solid
  - Mixed
- Secondary (with)
  - Fibrous dysplasia
  - Ossifying fibroma
  - Giant cell granuloma
  - Cementifying fibroma

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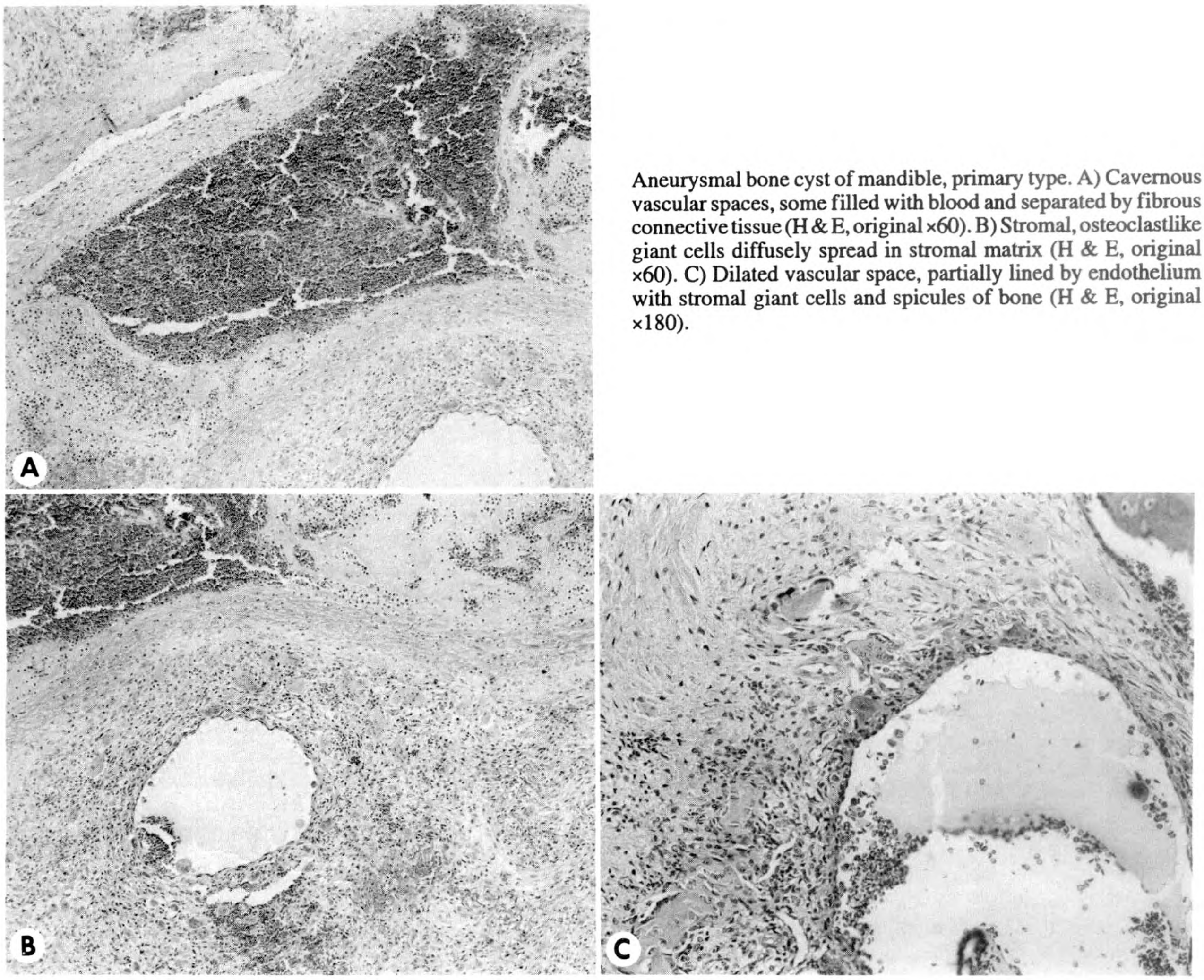

Aneurysmal bone cyst of mandible, primary type. A) Cavernous vascular spaces, some filled with blood and separated by fibrous connective tissue (H & E, original ×60). B) Stromal, osteoclastlike giant cells diffusely spread in stromal matrix (H & E, original ×60). C) Dilated vascular space, partially lined by endothelium with stromal giant cells and spicules of bone (H & E, original ×180).

Although the solid variant is unusual in the gnathic bones, its recognition is important because of the possibility of overcalling it as osteosarcoma. Vergel De Dios et al[2] give criteria for separating the two lesions.

Curettage with or without bone grafting is recommended for primary ABCs in non-gnathic bones. After such intralesional treatment, Vergel De Dios et al[2] report a 19% recurrence rate. Excision reduces this rate to virtually nil.[2] Reported recurrence rates for jaw lesions are 20% to 60% for curettage, 11% for excision, and as low as 8% with cryotherapy. Large lesions require complete surgical removal with reconstruction. Recurrent lesions tend to occur in patients younger than 20 years of age.[2] It is of interest that recurrences are rare more than 2 years postoperatively.[2]

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BOOK REVIEWS

Pathology of the Ear and Temporal Bone

George T. Nager. Hard cover, illustrated, indexed, 1,341 pages, 1993. Williams and Wilkins, Baltimore, Md.

This is a most impressive volume, written by an author known for his clarity of exposition. His training, not only as an otolaryngologist but as a pathologist, gives him a certain unique background for this work, as exemplified by the section on bone dysplasias. It is the culmination of his four decades of clinical and investigative otology.

The book is massive in content and so widely ranging that it could well be entitled "Pathology of the Ear and Skull Base."

The text contains 56 chapters, which are organized into 7 sections: I. Anatomy; II. Inflammatory Diseases; III. Benign, Questionably Malignant, Premalignant, and Malignant Neoplasms and Cysts; IV. Tumors of the Nerve Sheath, Meninges, Brain, and Other Space-occupying Lesions; V. Cystic Diseases of the Temporal Bone; VI. Bone Dysplasias; and VII. Other Disorders Involving the Ear and Temporal Bone. Chapters 15 to 21 in section III were written by Vincent J. Hyams, MD, Professor and Chairman Emeritus of the Department of Otolaryngic Pathology of the Armed Forces Institute of Pathology. Sections I, II, IV, and VI are so long and comprehensive that they constitute books within a book.

The volume is profusely illustrated with temporal bone and skull base photomicrographs, radiographs, and illustrations in color, half-tone, and pen and ink where a multiplane depiction is important to the message. The illustrations are almost all done by Leon Schlossberg, the star pupil of Johns Hopkins' famous medical illustrator, Max Brödel. A number of classic drawings of Brödel's are included, and they are still classics.

A number of sections stand out for their depth and comprehensiveness. Section I includes not only gross and microscopic anatomy in great detail, but also the dysplasias of the ear, anatomical variations, and anomalies. Section IV contains not only the schwannomas, but meningiomas, gliomas, chordomas, hemangiomas, leukemias, and plasmacytomas. The 8 chapters on bone dysplasias cover everything from otosclerosis to the craniotubular scleroses such as osteopathia striata and diaphyseal dysplasia.

The chapter on fractures and ballistic injuries alone makes this book of significant value to the general radiologist, neurosurgeon, emergency room physician, and forensic pathologist.

A striking coincidence occurred this month. Exactly 1 week before I received this volume for review, I received Harold F. Schuknecht's much-needed second edition of his *Pathology of the Ear*. As the two books were side by side on my table, a number of my colleagues asked me which one they should get. After having spent all my Christmas vacation reading them, my answer was: both. It is clear that they are not competitive, but complementary. I will keep them side by side on my reference shelf, and I urge every serious otologist to do the same.

Brian F. McCabe, MD
Iowa City, Iowa

Pathology of the Ear

Harold F. Schuknecht. Second edition. Hard cover, illustrated, indexed, 672 pages, 1993. Lea and Febiger, Philadelphia, Pa.

The long-awaited second edition of Dr Schuknecht's magnum opus is now available, to the delight of otologists around the world. It is not simply a tidied-up version of the first edition, but contains a great deal of new information on the pathology of the ear and clinical otology that he has accrued over the past 20 years. Not only has the number of specimens in the temporal bone bank of the Massachusetts Eye and Ear Infirmary grown considerably over this period (to 1,500), but the author expended considerable time and effort in personally visiting and studying other great temporal bone banks — 12 in Europe alone. He was aided by a virtual small army of otopathologists he trained from the ranks of his fellows and residents, many of whom now have world stature on their own.

The book retains the chapter on anatomy, but reduced in size, because much of the information is already available in a volume authored by Drs Schuknecht and A. Julianna Gulya, *Anatomy of the Temporal Bone With Surgical Implications*, published by Lea and Febiger in 1986. The number of chapters has been increased from 12 to 15 by adding 2 entirely new chapters: Disorders of the Immune System, and Surgical Pathology. Also, the first-edition chapter entitled Disorders of Growth, Metabolism and Aging has been split into 2 chapters — Disorders of Bone, and Inner Ear Disorders of Aging — because of the wealth of new knowledge in both of these areas.

The book is a handsome production that is profusely illustrated by extremely high-quality photomicrographs. If color is important to the message, the figure is in color. Signal structures or pathologic changes are labeled on the photograph itself, rather than buried in the legend, to enhance readability and comprehension. The legends are more extensive than those in the first edition and usually contain many features of the clinical history — an additional nuance. An important part of the book is its exhaustive bibliography, which not only demonstrates the author's scholarliness, but enables the reader to explore in depth a certain topic with little need to consult other sources.

It should be stressed that this volume is not simply on the pathology of the ear, but on the morphology of the temporal bone and its adjacent structures in health and disease, diagnostics, present and past treatments, and the natural history of the diseases discussed and illustrated. Thus, it is not an atlas, but a compendium. It should be on the ready reference shelf of every serious otologist.

Brian F. McCabe, MD
Iowa City, Iowa

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The Journal of Laryngology and Otology	11a
University Microfilms International	9a
Verona, University of	8a

Every precaution is taken to ensure accuracy, but we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

SUPRAX®

cefixime

ORAL SUSPENSION
100 mg / 5 mL
TABLETS
400 mg

Working 24 hours a day

rief Summary

UPRAX®
efixime
ral

Please see package insert for full Prescribing Information.

NDICATIONS AND USAGE

SUPRAX is indicated in the treatment of the following infections when caused by susceptible strains of the designated nicroorganisms:

ncomplicated Urinary Tract Infections caused by *Escherichia coli* and *Proteus mirabilis*.

titis Media caused by *Haemophilus influenzae* (beta-lactamase positive and negative strains), *Moraxella (Branamella) catarrhalis*, (most of which are beta-lactamase positive), and *Streptococcus pyogenes*.*

ote: For information on otitis media caused by *Streptococcus pneumoniae*, see **CLINICAL STUDIES** section.

haryngitis and Tonsillitis, caused by *S pyogenes*.

ote: Penicillin is the usual drug of choice in the treatment of *S pyogenes* infections, including the prophylaxis of rheumatic ever. SUPRAX is generally effective in the eradication of *S pyogenes* from the nasopharynx; however, data establishing the fficacy of SUPRAX in the subsequent prevention of rheumatic fever are not available.

cute Bronchitis and Acute Exacerbations of Chronic Bronchitis, caused by *S pneumoniae* and *H influenzae* (betactamase positive and negative strains).

ncomplicated Gonorrhea (Cervical/Urethral), caused by *Neisseria gonorrhoeae* (penicillinase- and nonpenicillinase-roducing strains).

Appropriate cultures and susceptibility studies should be performed to etermine the causative organism and its susceptibility to SUPRAX; hower, therapy may be started while awaiting the results of these studies. herapy should be adjusted, if necessary, once these results are known.

Efficacy for this organism in this organ system was studied in fewer than 0 infections.

LINICAL STUDIES

In clinical trials of otitis media in nearly 400 children between the ages f 6 months to 10 years, *S pneumoniae* was isolated from 47% of the atients, *H influenzae* from 34%, *M* (*B*) *catarrhalis* from 15%, and *pyogenes* from 4%.

The overall response rate of *S pneumoniae* to cefixime was approxinately 10% lower and that of *H influenzae* or *M* (*B*) *catarrhalis* approxinately 7% higher (12% when beta-lactamase positive strains of *influenzae* are included) than the response rates of these organisms to e active control drugs.

In these studies, patients were randomized and treated with either efixime at dose regimens of 4 mg/kg BID or 8 mg/kg QD, or with a tandard antibiotic regimen. Sixty-nine percent to 70% of the patients in ach group had resolution of signs and symptoms of otitis media when valuated 2 to 4 weeks posttreatment, but persistent effusion was found 15% of the patients. When evaluated at the completion of therapy, 17% of patients receiving cefixime and 14% of patients ceiving effective comparative drugs (18% including those patients who had *H influenzae* resistant to the control drug and ho received the control antibiotic) were considered to be treatment failures. By the 2- to 4-week follow-up, a total of 0% to 31% of patients had evidence of either treatment failure or recurrent disease.

Convenient QD Dosing

One 400 mg Tablet/day

Oral Suspension (8 mg/kg/day)

(For patients older than 12 years.)

Bacteriological Outcome of Otitis Media at 2 to 4 Weeks Posttherapy
Based on Repeat Middle Ear Fluid Culture or
Extrapolation from Clinical Outcome

rganism	Cefixime[a] 4 mg/kg BID	Cefixime[a] 8 mg/kg QD	Control[a] drugs
treptococcus pneumoniae	48/70 (69%)	18/22 (82%)	82/100 (82%)
aemophilus influenzae beta-lactamase negative	24/34 (71%)	13/17 (76%)	23/34 (68%)
aemophilus influenzae beta-lactamase positive	17/22 (77%)	9/12 (75%)	1/1[b]
oraxella (Branhamella) catarrhalis	26/31 (84%)	5/5	18/24 (75%)
pyogenes	5/5	3/3	6/7
ll Isolates	120/162 (74%)	48/59 (81%)	130/166 (78%)

Number eradicated/number isolated.

An additional 20 beta-lactamase positive strains of *H influenzae* were isolated, but were excluded from this analysis because they were resistant to the control antibiotic. In 19 of these, the clinical course could be assessed, and a favorable outcome occurred in 10. When these cases are included in the overall bacteriological evaluation of therapy with the control drugs, 140/185 (76%) of pathogens were considered to be eradicated.

Tablets should not be substituted for suspension when treating otitis media.

ONTRAINDICATIONS

SUPRAX is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

ARNINGS

BEFORE THERAPY WITH SUPRAX IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE HETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, R OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE XERCISED BECAUSE CROSS HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC EACTION TO SUPRAX OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES, AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Administer cautiously to allergic patients.

Treatment with broad-spectrum antibiotics, including SUPRAX, alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of severe antibiotic-associated diarrhea including pseudomembranous colitis.

Pseudomembranous colitis has been reported with the use of SUPRAX and other broad-spectrum antibiotics (including macrolides, semisynthetic penicillins, and cephalosporins); therefore, it is important to consider this diagnosis in patients who develop diarrhea in association with the use of antibiotics. Symptoms of pseudomembranous colitis may occur during or after antibiotic treatment and may range in severity from mild to life-threatening. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, management should include fluids, electrolytes, and protein supplementation. If the colitis does not improve after the drug has been discontinued, or if the symptoms are severe, oral vancomycin is the drug of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes of colitis should be excluded.

PRECAUTIONS

General: Use, especially when prolonged, may result in overgrowth of resistant organisms. If superinfection occurs during therapy, take appropriate measures.

Carefully monitor patients on dialysis. Adjust dosage of SUPRAX in patients with renal impairment and those undergoing continuous ambulatory peritoneal dialysis and hemodialysis. (See **DOSAGE AND ADMINISTRATION** in package insert.)

Prescribe cautiously in patients with a history of gastrointestinal disease, particularly colitis.

Drug Interactions: No significant drug interactions have been reported to date.

Drug/Laboratory Test Interactions: A false-positive reaction for ketones in the urine may occur with tests using nitroprusside but not with those using nitroferricyanide.

SUPRAX administration may result in a false-positive reaction for glucose in the urine using Clinitest®,** Benedict's solution, or Fehling's solution. Use glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix®** or Tes-Tape®**).

A false-positive direct Coombs test has been reported during treatment with other cephalosporin antibiotics; therefore, it should be recognized that a positive Coombs test may be due to the drug.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although no lifetime animal studies have been conducted to evaluate carcinogenic potential, no mutagenic potential of SUPRAX was found in standard laboratory tests. In rats, reproductive studies revealed no fertility impairment at doses up to 125 times the adult therapeutic dose.

Usage in Pregnancy: *Pregnancy Category B:* Reproduction studies have been performed in mice and rats at doses up to 400 times the human dose and have revealed no evidence of harm to the fetus due to SUPRAX. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: SUPRAX has not been studied for use during labor and delivery. Treatment should only be given if clearly needed.

Nursing Mothers: It is not known whether SUPRAX is excreted in human milk. Consider discontinuing nursing temporarily during treatment with this drug.

Pediatric Use: Safety and effectiveness of SUPRAX in children aged less than 6 months have not been established.

The incidence of gastrointestinal adverse reactions, including diarrhea and loose stools, in pediatric patients receiving the suspension was comparable to that seen in adult patients receiving tablets.

ADVERSE REACTIONS

Most adverse reactions observed in clinical trials were of a mild and transient nature. Five percent (5%) of patients in the US trials discontinued therapy because of drug-related adverse reactions. The most commonly seen adverse reactions in US trials of the tablet formulation were gastrointestinal events, which were reported in 30% of adult patients on either the BID or the QD regimen. Clinically mild gastrointestinal side effects occurred in 20% of all patients, moderate events occurred in 9% of all patients, and severe adverse reactions occurred in 2% of all patients. Individual event rates included diarrhea 16%, loose or frequent stools 6%, abdominal pain 3%, nausea 7%, dyspepsia 3%, and flatulence 4%. The incidence of gastrointestinal adverse reactions, including diarrhea and loose stools, in pediatric patients receiving the suspension was comparable to that seen in adult patients receiving tablets.

These symptoms usually responded to symptomatic therapy or ceased when SUPRAX was discontinued.

Several patients developed severe diarrhea and/or documented pseudomembranous colitis, and a few required hospitalization.

The following adverse reactions have been reported following the use of SUPRAX. Incidence rates were less than 1 in 50 (less than 2%), except as noted above for gastrointestinal events.

Gastrointestinal: Diarrhea, loose stools, abdominal pain, dyspepsia, nausea, and vomiting. Several cases of documented pseudomembranous colitis were identified during the studies. The onset of pseudomembranous colitis symptoms may occur during or after therapy.

Hypersensitivity Reactions: Skin rashes, urticaria, drug fever, and pruritus. Erythema multiforme, Stevens-Johnson syndrome, and serum sickness-like reactions have been reported.

Hepatic: Transient elevations in SGPT, SGOT, and alkaline phosphatase.

Renal: Transient elevations in BUN or creatinine.

Central Nervous System: Headaches or dizziness.

Hemic and Lymphatic Systems: Transient thrombocytopenia, leukopenia, and eosinophilia. Prolongation in prothrombin time was seen rarely.

Other: Genital pruritus, vaginitis, candidiasis.

The following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics:

Adverse Reactions: Allergic reactions including anaphylaxis, toxic epidermal necrolysis, superinfection, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemolytic anemia, hemorrhage, and colitis.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see **DOSAGE AND ADMINISTRATION** and **OVERDOSAGE**). If seizures associated with drug therapy occur, discontinue drug. Administer anticonvulsant therapy if clinically indicated.

Abnormal Laboratory Tests: Positive direct Coombs test, elevated bilirubin, elevated LDH, pancytopenia, neutropenia, agranulocytosis.

OVERDOSAGE

Gastric lavage may be indicated; otherwise, no specific antidote exists. Cefixime is not removed in significant quantities from the circulation by hemodialysis or peritoneal dialysis. Adverse reactions in small numbers of healthy adult volunteers receiving single doses up to 2 g of SUPRAX did not differ from the profile seen in patients treated at the recommended doses.

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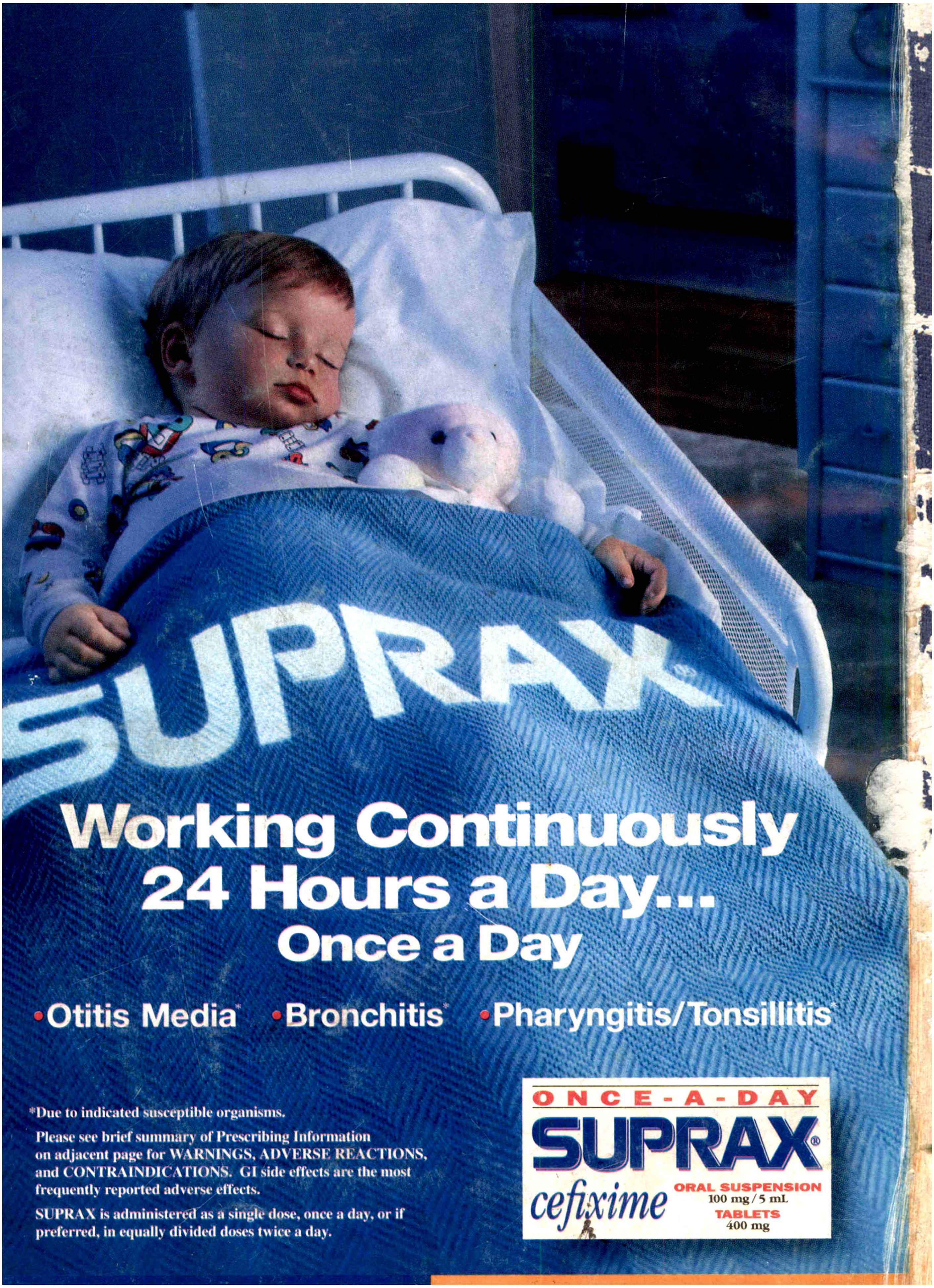

Working Continuously 24 Hours a Day... Once a Day

- Otitis Media*
- Bronchitis*
- Pharyngitis/Tonsillitis*

*Due to indicated susceptible organisms.

Please see brief summary of Prescribing Information on adjacent page for WARNINGS, ADVERSE REACTIONS, and CONTRAINDICATIONS. GI side effects are the most frequently reported adverse effects.

SUPRAX is administered as a single dose, once a day, or if preferred, in equally divided doses twice a day.

ONCE-A-DAY
SUPRAX®
cefixime
ORAL SUSPENSION
100 mg / 5 mL
TABLETS
400 mg